		Inventaris Wob-verzoek W17-07								
			wordt verstrekt				**weigeringsgronden**			
nr.		**document NTS 2016806**	**reeds openbaar**	**niet**	**geheel**	**deels**	**10.1.c**	**10.2.e**	**10.2.g**	**11.1**
1		Aanvraagformulier				x		x		
2		NTS	x							
3		Project proposal				x	x	x	x	
4		Bijlage animal procedure 1				x	x	x	x	
5		Bijlage animal procedure 2				x	x	x	x	
6		Bijlage animal procedure 3				x	x	x	x	
7		DEC advies				x		x	x	
8		Aanvullende informatie				x		x	x	
9		Advies CCD aan bestuur		x						x
10		Beschikking				x		x	x	

06 JAN 2017

Aanvraag
Projectvergunning Dierproeven
Administratieve gegevens

- U bent van plan om één of meerdere dierproeven uit te voeren.
- Met dit formulier vraagt u een vergunning aan voor het project dat u wilt uitvoeren. Of u geeft aan wat u in het vergunde project wilt wijzigen.
- Meer informatie over de voorwaarden vindt u op de website www.zbo-ccd.nl of in de toelichting op de website.
- Of bel met 0900-2800028 (10 ct/min).

1 Gegevens aanvrager

1.1 Heeft u een deelnemernummer van de NVWA?
Neem voor meer informatie over het verkrijgen van een deelnemernummer contact op met de NVWA.

☑ Ja > *Vul uw deelnemernummer in* 10300 2016106

☐ Nee > *U kunt geen aanvraag doen*

1.2 Vul de gegevens in van de instellingsvergunninghouder die de projectvergunning aanvraagt.

Naam instelling of organisatie	Stichting Katholieke Universiteit Nijmegen
Naam van de portefeuillehouder of diens gemachtigde	Instantie voor dierenwelzijn
KvK-nummer	4 1 0 5 5 6 2 9

1.3 Vul de gegevens van het postadres in.
Alle correspondentie van de CCD gaat naar de portefeuillehouder of diens gemachtigde en de verantwoordelijke onderzoeker.

Straat en huisnummer	Geert Grooteplein 10
Postbus	9101, [redacted]
Postcode en plaats	6500HB Nijmegen
IBAN	NL90ABNA0231209983
Tenaamstelling van het rekeningnummer	UMC St Radboud

1.4 Vul de gegevens in van de verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	☐ Dhr. ☑ Mw. [redacted]
Functie	[redacted]
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.5 *(Optioneel)* Vul hier de gegevens in van de plaatsvervangende verantwoordelijke onderzoeker.

(Titel) Naam en voorletters	☐ Dhr. ☑ Mw. [redacted]
Functie	PhD student
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.6 *(Optioneel)* Vul hier de gegevens in van de persoon die er verantwoordelijk voor is dat de uitvoering van het project in overeenstemming is met de projectvergunning.

(Titel) Naam en voorletters	[redacted] ☐ Dhr. ☐ Mw.
Functie	Instantievoor Dierenwelzijn
Afdeling	[redacted]
Telefoonnummer	[redacted]
E-mailadres	[redacted]

1.7 Is er voor deze projectaanvraag een gemachtigde?

☑ Ja > *Stuur dan het ingevulde formulier Melding Machtiging mee met deze aanvraag*

☐ Nee

2 Over uw aanvraag

2.1 Wat voor aanvraag doet u?

☒ Nieuwe aanvraag > Ga verder met vraag 3

☐ Wijziging op (verleende) vergunning die negatieve gevolgen kan hebben voor het dierenwelzijn

Vul uw vergunde projectnummer in en ga verder met vraag 2.2

☐ Melding op (verleende) vergunning die geen negatieve gevolgen kan hebben voor het dierenwelzijn

Vul uw vergunde projectnummer in en ga verder met vraag 2.3

2.2 Is dit een *wijziging* voor een project of dierproef waar al een vergunning voor verleend is?

☐ Ja > Beantwoord dan in het projectplan en de niet-technische samenvatting alleen de vragen waarop de wijziging betrekking heeft en onderteken het aanvraagformulier

☑ Nee > Ga verder met vraag 3

2.3 Is dit een *melding* voor een project of dierproef waar al een vergunning voor is verleend?

☑ Nee > Ga verder met vraag 3

☐ Ja > Geef hier onder een toelichting en ga verder met vraag 6

3 Over uw project

3.1 Wat is de geplande start- en einddatum van het project?

Startdatum	29-01-2017
Einddatum	28-01-2022

3.2 Wat is de titel van het project?

[redacted] matters! [redacted] effect

3.3 Wat is de titel van de niet-technische samenvatting?

Nieuwe inzichten in de rol van serotonine in ontwikkelingsstoornissen.

3.4 Wat is de naam van de Dierexperimentencommissie (DEC) aan wie de instellingsvergunninghouder doorgaans haar projecten ter toetsing voorlegt?

Naam DEC	RU DEC
Postadres	Postbus 9101, 6500 HB Nijmegen [redacted]
E-mailadres	[redacted]

4 Betaalgegevens

4.1 Om welk type aanvraag gaat het?

- ☑ Nieuwe aanvraag Projectvergunning € 1.441,00 Lege
- ☐ Wijziging € Lege

4.2 Op welke wijze wilt u dit bedrag aan de CCD voldoen.
Bij een eenmalige incasso geeft u toestemming aan de CCD om eenmalig het bij 4.1 genoemde bedrag af te schrijven van het bij 1.2 opgegeven rekeningnummer.

- ☐ Via een eenmalige incasso
- ☑ Na ontvangst van de factuur

5 Checklist bijlagen

5.1 Welke bijlagen stuurt u mee?

Verplicht

- ☒ Projectvoorstel
- ■ Niet-technische samenvatting

Overige bijlagen, indien van toepassing

- ☐ Melding Machtiging
- ☒ DEC-advies en factuurinformatie

6 Ondertekening

6.1 Print het formulier uit, onderteken het en stuur het inclusief bijlagen via de beveiligde e-mailverbinding naar de CCD of per post naar:

Centrale Commissie
Dierproeven
Postbus 20401
2500 EK Den Haag

Ondertekening door de instellingsvergunninghouder of gemachtigde (zie 1.6). De ondergetekende verklaart:

- dat het projectvoorstel is afgestemd met de Instantie voor Dierenwelzijn.
- dat de personen die verantwoordelijk zijn voor de opzet van het project en de dierproef, de personen die de dieren verzorgen en/of doden en de personen die de dierproeven verrichten voldoen aan de wettelijke eisen gesteld aan deskundigheid en bekwaamheid.
- dat de dieren worden gehuisvest en verzorgd op een wijze die voldoet aan de eisen die zijn opgenomen in bijlage III van richtlijn 2010/63/EU, behalve in het voorkomende geval de in onderdeel F van de bijlage bij het bij de aanvraag gevoegde projectvoorstel gemotiveerde uitzonderingen.
- dat door het ondertekenen van dit formulier de verplichting wordt aangegaan de leges te betalen voor de behandeling van de aanvraag.
- dat het formulier volledig en naar waarheid is ingevuld.

Naam	[redacted]
Functie	Instantie voor dierenwelzijn
Plaats	Nijmegen
Datum	29-12-2016
Handtekening	[redacted]

Form
Project proposal

- This form should be used to write the project propsal of animal procedures.
- The appendix 'description animal procedures' is an appendix to this form. For each type of animal procedure, a separate appendix 'description animal procedures' should be enclosed
- For more information on the project proposal, see our website(www.zbo-ccd.nl).
- Or contact us by phone (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen
1.3	Provide the title of the project.	[redacted] matters! [redacted] effects

2 Categories

2.1 Please tick each of the following boxes that applies to your project.

[X] Basic Research
[] Translational or applied research
[] Regulatory use of routine production
[] Research into environmental protection in the interest of human or animal health or welfare dier
[] Research aimed at preserving the species subjected to procedures

[] Higher education or training
[] Forensic enquiries
[] Maintenance of colonies of genetically altered animals not used in other animal procedures

3 General description of the project

3.1 Background

Describe the project (motivation, background and context) with respect to the categories selected in 2.

- For legally required animal procedures, indicate which statutory or regulatory requirements apply (with respect to the intended use and market authorisation).
- For routine production, describe what will be produced and for which uses.
- For higher education or training, explain why this project is part of the educational program and describe the learning targets.

Neurodevelopmental disorders

Neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADHD) and autism are gene x environmental disorders which manifest in early development. These disorders are characterized by developmental deficits resulting in a variety of impaired cognitive and social functions (American Psychiatric Association, 2013).

ADHD is the third most common childhood chronic health condition with a prevalence of 3.4% in children (Polanczyk et al., 2015). ADHD is characterized by inattention, hyperactivity and impulsiveness and has a strong genetic basis. In 60 to 70% of the childhood diagnostics, ADHD persists into adulthood (Kessler et al., 2005). Autism has a prevalence of approximately 1.0% (Lai et al., 2014). Autism is characterized by early-onset difficulties in social communication and social interaction; and shows restricted and repetitive behavior, interests or activities. Both disorders are more prevalent in males with a male:female sex ratio of 3:1 to 16:1 in ADHD and a ratio of 2:1 to 3:1 in autism (Lai et al., 2014; Novik et al., 2006). During childhood these disorders highly overlap, for example 28-44% of autism patients are affected with ADHD-related symptoms (Lai et al., 2014). This means that children diagnosed with autism often show ADHD-related symptoms and vice versa for children diagnosed with ADHD. Moreover, ADHD and autism may share common genetic liability (Karalunas et al., 2014).

Noteworthy, ADHD and autism often co-occur with other psychiatric conditions such as anxiety, depression and obsessive-compulsive disorder (OCD). Children with autism have a prevalence of 42-56% to develop anxiety, 12-70% towards depression and 7-24% towards OCD (Lai et al., 2014). Noteworthy, it is possible that co-occurrence of depression is only detectable during adolescence or adulthood (Lai et al., 2014). Children with ADHD have a prevalence of 27% to develop anxiety, 13% towards depression and 8% towards OCD (overview in Singh et al., 2015; Geller et al., 2000). Interestingly however, as discussed by the World Health Organization (http://www.who.int/mental_health/prevention/genderwomen/en/) gender is a critical determinant of mental health and mental illness including depression. Depression is not only twice as common in women but seems to be more persistent in women as well.

The high co-occurrence rate between ADHD, autism, anxiety, depression and OCD can be the result of shared pathophysiology. Given this current state there is a high demand for a better understanding of the biological mechanisms underlying these psychiatric diseases.

in disorders

(which is a molecule) derived from and is mainly found in the and In the central nervous system, certain , i.e. the cell bodies of the into the brain. It is involved in many biological processes such as learning and memory, attention, impulse control, mood and social behavior and seems to be involved in a variety of psychiatric disorders (Berger et al., 2008).
There are two major factors regulating :

., These variations result in different which means that the activity of the variants can lead to different in the blood. For example, correlations have been observed between the table 1.

Table 1. variations linked to

The old hypothesis,

The [redacted] hypothesis regarding the role of [redacted] in [redacted] disorders is that [redacted] as a [redacted] is responsible for the development of [redacted] disorder(s) [redacted]
The elevated [redacted] levels in the blood, observed for the first time in 1961, is used as the most robust biomarker for the [redacted] in autism: the [redacted] state. Nevertheless, literature is still [redacted] autism studies show also data supporting the [redacted] for autism (summarized by [redacted] and data concerning the role of [redacted] in ADHD also favors both [redacted] summarized by [redacted] These [redacted] hypotheses ask for a deeper understanding [redacted]

[redacted]

During [redacted] are located in the [redacted] whereas both the [redacted] are located in the [redacted] Research using experimental animals has revealed that the [redacted] to [redacted]. [redacted] projection neurons reach the [redacted]). Hence, only [redacted]. This finding is in line with human observations, showing that after [redacted] This means that a child can synthesize [redacted] only independently [redacted]. Furthermore, between [redacted] is temporarily expressed on [redacted], presumably to [redacted]

[redacted]). Later in life, when the brain is mature and neurons have migrated to their final destination, [redacted]). The start of the synthetization of [redacted] and the [redacted]

While [redacted] production starts [redacted], [redacted] of the [redacted] as well. Important research of [redacted] et al. showed in [redacted] that [redacted] from the [redacted] In figure 1 this [redacted].
As the fetus, and in particular its [redacted] is largely dependent on [redacted] figure 1) ([redacted] et al., [redacted] we hypothesize that the [redacted]. Hence, [redacted] allows us to differentiate between [redacted].

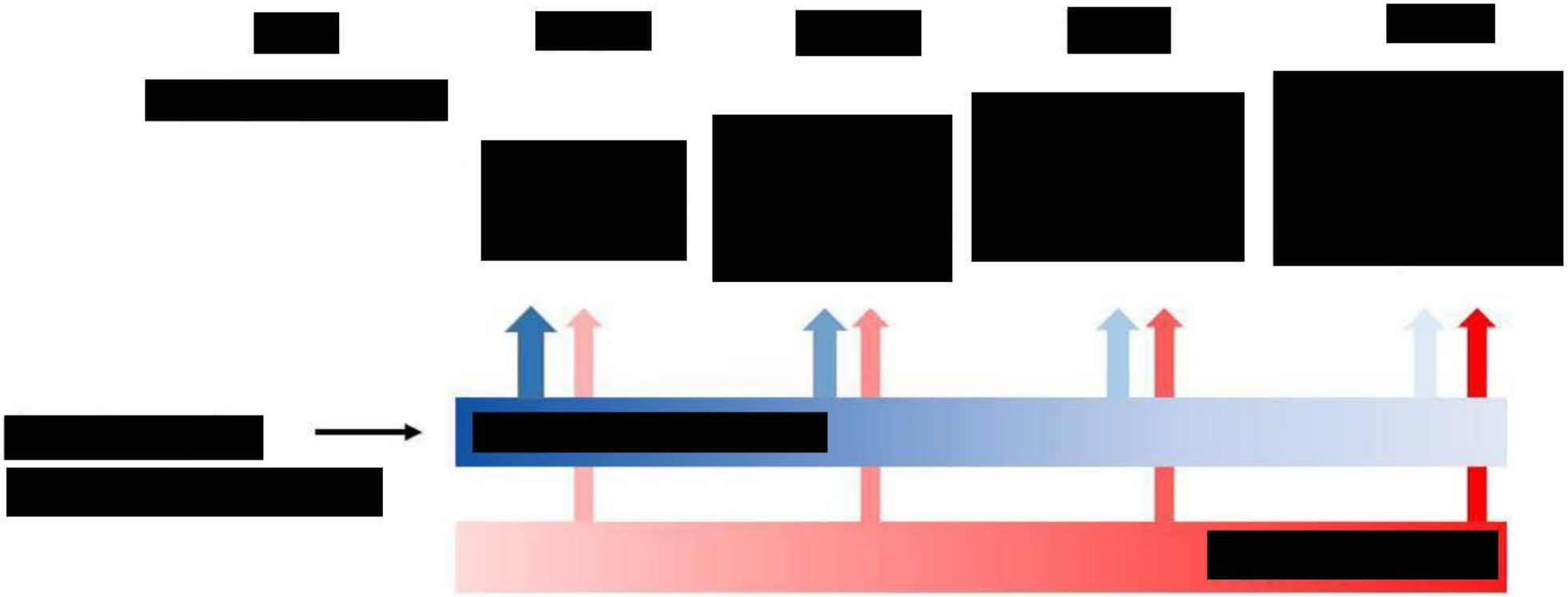

Figure 1. **Two [redacted].**
In rodents the source of [redacted] in the [redacted]

[redacted] source. [redacted]

For this reason, [redacted] in humans and [redacted] are likely associated with variation in [redacted] levels during early neurodevelopment through the [redacted] For this reason, pups with a [redacted] one of these [redacted] [redacted] [redacted]) are at risk to develop [redacted] disorders.
[redacted] recently conducted a human pilot study and reported that [redacted] affected the development of children. [redacted] compared MRI scans and cognitive performance of N=35 children of [redacted] to those of N=44 children of [redacted] All children themselves carried the [redacted] [redacted] compared to [redacted], thereby showing that the [redacted] can affect [redacted]
In another human pilot study [redacted] carriers and 41 of their [redacted] revealed that offspring of [redacted] exhibited 1.5- to 2.5-times-higher ADHD scores and related symptoms than did controls or offspring of fathers with the corresponding [redacted] Moreover, a recent article of [redacted],
[redacted].
Focusing on animal data, mouse [redacted] that were fully capable of producing [redacted] but conceived by [redacted], had an [redacted] More importantly, recent studies from [redacted] his research group [redacted] showed in mice that the [redacted] has an effect on [redacted] and [redacted] and influences [redacted].
Thus [redacted] has profound effects on [redacted] in [redacted] However, what the underlying processes are is currently unknown. Noteworthy, Johansson et al. (2010) did not find evidence linking [redacted] within [redacted] themselves to ADHD.

[redacted]

Both [redacted] thereby potentially affecting [redacted]

- indirectly influence [redacted]
- influence the [redacted]
- influence the [redacted] which consequently influence [redacted]

[redacted]

- directly [redacted]
- indirectly [redacted]

[redacted]

Part of the [redacted]. Therefore, [redacted] and thereby affect [redacted]

- affects [redacted]
- affects [redacted]
- directly [redacted]
- indirectly [redacted]

An example of a [redacted] is the [redacted]. In [redacted] showed that [redacted] have an increase in [redacted] resulting in an [redacted] As [redacted] is involved in [redacted] cell survival and differentiation, these alterations can potentially affect [redacted].
In sum, these proposed routes of the influence of [redacted] on the [redacted] can lead to altered [redacted] function and/or variation in [redacted] supply and subsequently altered [redacted]. Again, these [redacted] may result in [redacted] in [redacted] and thus potentially an enhanced liability to [redacted].

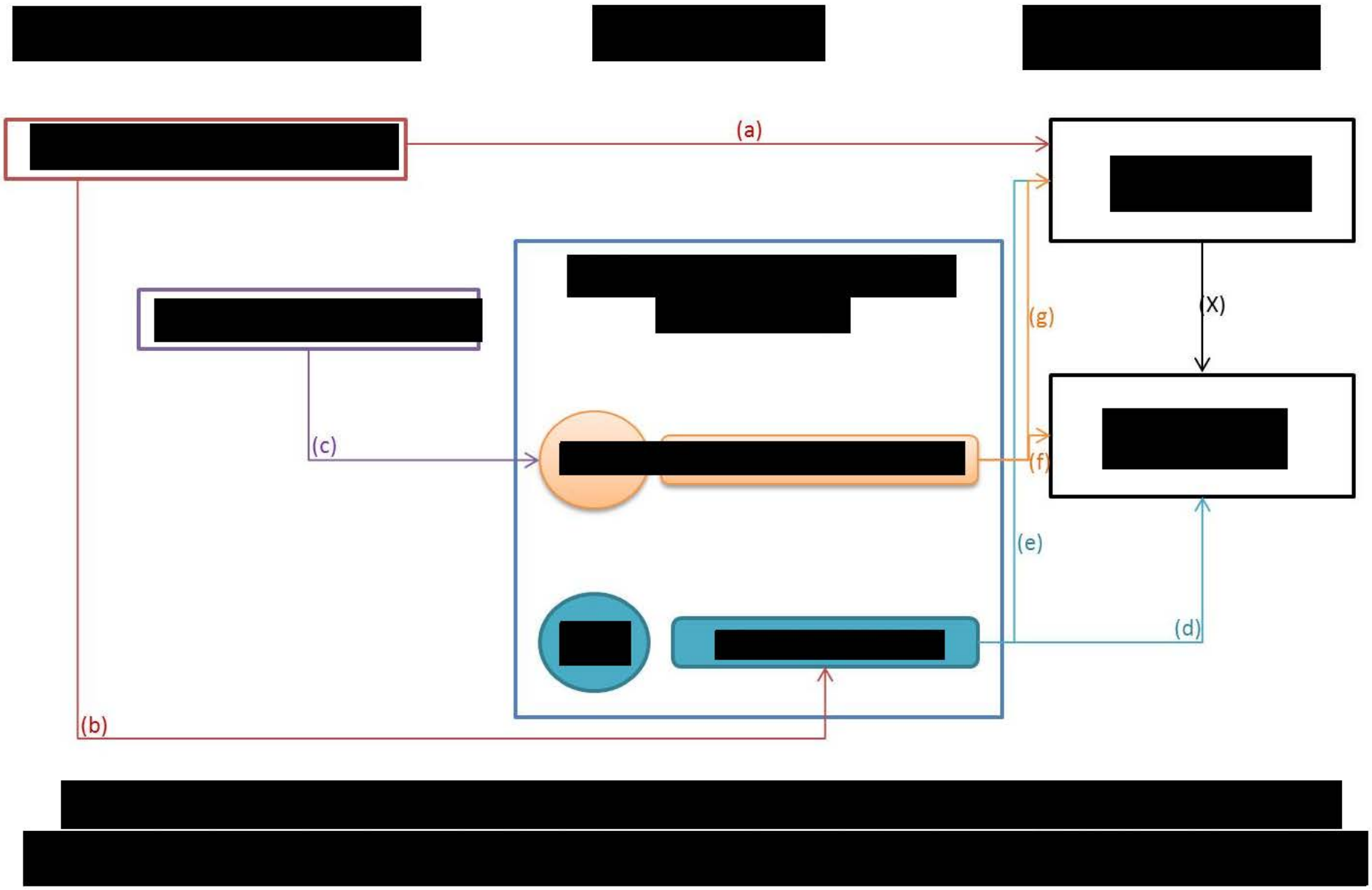
(a)
(X)
(g)
(c)
(f)
(e)
(d)
(b)

Figure 2. Three potential
Three routes by which

There are some differences between rats / and humans. First of all,
. However, both . Both ,
In both
Moreover, the depth of
For this reason, the rat is a good model for

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[redacted]

3.2 Purpose

Describe the project's main objective and explain why this objective is achievable.

- If the project is focussed on one or more research objectives, which research questions should be addressed during this project?
- If the main objective is not a research objective, which specific need(s) does this project respond to?

The overall aim of this study is to investigate the [redacted] hypothesis of [redacted] in a rigorous way targeting three research questions:
1) Which of the [redacted]
2) How is the [redacted]
3) What are the effects of [redacted]

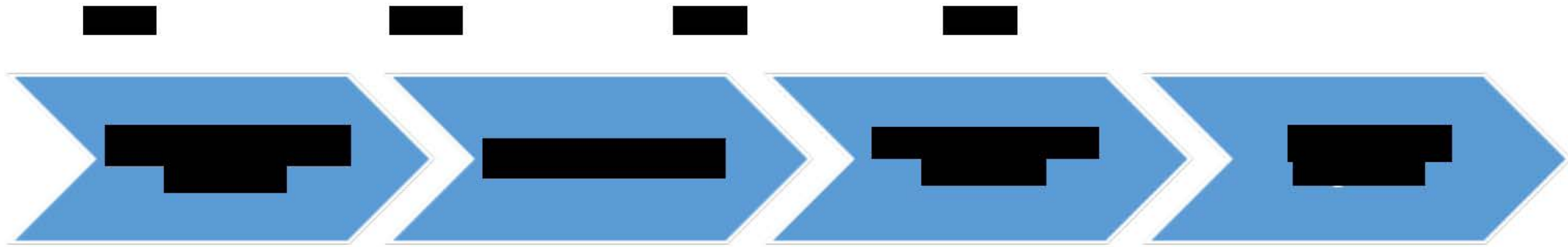


Figure 3. Research questions

Feasibility

The [redacted] rat was designed in the current laboratory and [redacted] has studied the behavioral and neurochemical characteristics of this [redacted] rat for over more than 10 years. Hence, [redacted] has the needed experience and facilities to perform the above mentioned studies [redacted]). The [redacted] rat is ordered and is expected to arrive in [redacted] in the beginning of 2017. Our experience with behavioral and cellular/molecular measurements in rats, together with the availability of the required equipment and the expertise of how to apply these, provides high feasibility of this project.

Reference

[redacted]

[redacted]

3.3 Relevance

What is the scientific and/or social relevance of the objectives described above?

Scientific relevance

In this project we will examine through which underlying mechanisms [redacted] effects increase the risk towards [redacted] disorders. The data will lead to an increase in [redacted] understanding of [redacted] predisposition through the [redacted]

[redacted] findings may lead to a substantial paradigm shift in psychiatric genetics because the results would imply that [redacted]. Consequently, the [redacted] has to be taken into account in [redacted] to increase [redacted] understanding of [redacted] to [redacted]. This paradigm shift will lead to new research into the [redacted] for other [redacted]

Societal relevance

The prevalence for ADHD is calculated to be 3.4%; the third most common childhood chronic health condition (Polanczyk et al., 2015). At the moment various treatment possibilities are available, however they all have their limitations as shown for ADHD by Childress & Tran (2016): low efficacy; side effects; treatment gaps. [redacted] research could open up a new area of research into other factors (like [redacted]) that co-determine the variation in plasma [redacted] levels and thereby [redacted] in the [redacted], and into possible strategies (e.g. diet, lifestyle) that modify or remediate [redacted] risk factors for abnormal variation in early [redacted] metabolism. This understanding will be ultimately crucial for the design of more effective strategies to treat these disorders by modifying and/or remediating risk factors for abnormal variation in early [redacted] metabolism. This can potentially lead towards novel prevention approaches, in a personalized manner. This may in the future have beneficial effects not only for patients, but also for the other members of our society.

Reference

[redacted]

Childress, A., & Tran, C. (2016). Current Investigational Drugs for the Treatment of Attention-Deficit/Hyperactivity Disorder. Expert Opinion on Investigational Drugs (Vol. 3784). http://doi.org/10.1517/13543784.2016.1147558

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., & Rohde, L. A. (2015). Annual research review: A meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *Journal of Child Psychology and Psychiatry and Allied Disciplines*, *56*(3), 345–365. http://doi.org/10.1111/jcpp.12381

3.4 Research Strategy

3.4.1 Provide an overview of the overall design of the project (strategy).

To answer the research questions described under 3.2. we will make use of two rat models; [redacted] rat.
The effects of the [redacted] variant can be mimicked by using null mutant [redacted] rats. Both types of these [redacted] altered rat models exhibit [redacted] levels ([redacted]); mimicking *h*[redacted]. [redacted] rats display [redacted] in the [redacted]). While [redacted] animals are often used because of their [redacted], from a [redacted] perspective, [redacted] rats are considered as the [redacted] model for [redacted] because the [redacted] carriers display a [redacted] rather than a complete [redacted] For this reason, both [redacted] and [redacted] models represent the [redacted] variant and will both be studied: [redacted] in study 1 and [redacted] in study 2.
By using null mutant [redacted] or [redacted] rats the effects of the [redacted] can be mimicked, resulting in [redacted] levels in the blood and thereby mimicking [redacted] showed in mice that [redacted] of [redacted] mice are indeed significantly decreased to 3.4% of [redacted] levels in the gut and 8% of [redacted] levels in the blood.
To answer research question 1 and 2, both animal models (the [redacted] and the [redacted]) need to be studied during pregnancy at two different time-points; a fetus dependent on [redacted] and a fetus dependent on [redacted]. To answer research question 3, the [redacted] needs to be studied over time to [redacted] In this way it is possible to investigate which [redacted] correlates to the ADHD/autism-related or co-morbid-related behavior, brain structural & functional traits. Moreover, this makes it possible to investigate which [redacted] changes are underlying ADHD/autism-related or co-morbid-related behavior, brain structural & functional traits. [redacted] will answer the research questions by using two studies. In the first study, the robust animal models are used (i.e. [redacted]). If the results show significant differences between [redacted] in comparison to [redacted] rats, [redacted] will proceed with investigating [redacted] rats ([redacted]). This process is depicted in figure 4.

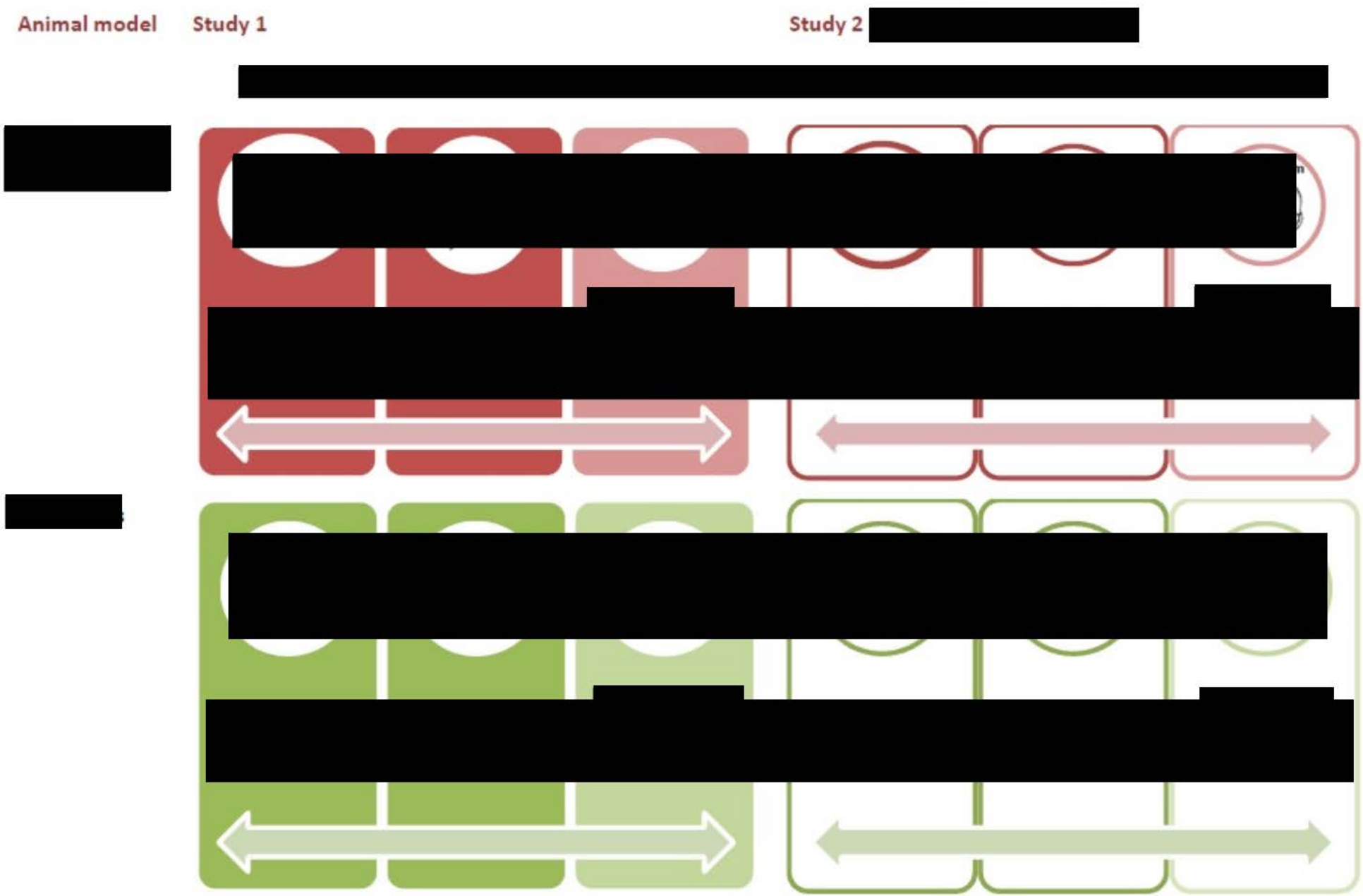

Animal model
Study 1
Study 2
[redacted]

Figure 4. Research project strategy.
For each animal model mimicking [redacted] four research questions need to be answered related to [redacted], [redacted] and ADHD/autism-related behavioral and brain structural & functional traits. Therefore, two experiments need to be performed, 1) [redacted] focusing on [redacted] (dark red/green); and 2) throughout life focusing on psychiatric disease-related behavioral and brain structural & functional traits with a main focus on ADHD/autism (light red/green). These experiments will be studied in [redacted] rats. If this model supports the [redacted] a second study starts investigating if these effects are visible in [redacted]) rats in comparison to [redacted] rats (go / no-go moment).

The project.
Beside the two studies (study 1 and 2) [redacted] first need to investigate if [redacted] is affected by [redacted] and a pilot study needs to be performed to investigate if the [redacted] holds in rat models. This is shown in a flowchart in figure 5. All parts of the project are explained in more detail below.


Pilot studies
experiments
per group)
Pilot studies
experiments
per group)
study
group)
function
study
group)
study
group)
study
group)
study
group)
study
group)
study
group)
study 2
group)
study
group)
OCD2 study
group)
study 2
group)
study 2
group)
function study
group)
study
group)
per group)

Figure 5. Flowchart project.
For both animal models, [redacted], first a [redacted] study will be performed to investigate if the [redacted] differs due to the [redacted]). If [redacted] differs cross-fostering needs to be performed meaning that half of the pups will go to a foster mother leading to an increase in amount of pups. For both animal models the decision of [redacted] is taken separately; when [redacted] is needed the purple-pathway will be performed, whereas, if [redacted] is not needed the blue-pathway will be performed. After this decision a pilot study will be performed with two experiments: an [redacted] study investigating [redacted] changes during [redacted] and a [redacted] study investigating autistic-related traits. At the first go / no-go moment (red line), a 'go' is given when one of the experiments show significant differences between pups of [redacted]; this is decided for each animal model separately. After the go-moment 'study 1' is performed (the experiments between the red lines). At the second go / no-go moment (second red line) a 'go' is given for each experiment separately: when an experiment shows significant differences between [redacted] 'study 2' will be performed investigating pups of [redacted] (this decision will be made for both animal models and for both genders separately). Importantly for the purple-pathway: before 'study 2' can be performed another [redacted] study need to be performed to decide if the [redacted] between [redacted] differs as well. If between [redacted] and [redacted] no differences in care is found no [redacted] is needed in 'study 2'.

[redacted] **(DAP 3)**
It is commonly known that [redacted] can influence offspring's behavior and psychiatric liability. Thus, if [redacted] influences [redacted] this can affect our results.
Therefore, [redacted] will study the [redacted] in our studies.
After delivery, [redacted] will be scored to investigate if there is a significant difference in [redacted] behavior due to [redacted] or [redacted] differences. If there is a significant difference in [redacted] and [redacted] and/or [redacted], [redacted] will be applied in all the groups in DAP 3, meaning that [redacted]. This is explained in DAP 3: A2 in more detail. If there is no significant difference between [redacted] mothers and [redacted] mothers, the pups will not be cross fostered.

[redacted] **study (DAP 3)**
As the levels of [redacted] in [redacted] can differ between the [redacted], the [redacted] levels in [redacted] of [redacted] need to be measured.
This study will only be performed when [redacted] to be executed and when pilot study results in a 'go'.

Pilot studies (DAP 1)
As the evidence for the [redacted] comes from human and mice data, first two pilot experiments will be performed to investigate if the [redacted] are also seen in these rat models.
As shown in mice and human data [redacted] ***actions can result in*** [redacted]

1. [redacted] experiment
In the [redacted] experiment we will focus on the findings of mice data showing altered [redacted] (findings of [redacted]

Animal models

[redacted] animal model

. [redacted]

. [redacted]

[redacted] animal model:

. [redacted]

. [redacted]

Methods

These [redacted] will be sacrificed by decapitation at [redacted] to study [redacted] and to study [redacted] changes. The [redacted] changes [redacted] want to investigate are the balance between excitatory and inhibitory neurons using fluorescence immunohistochemistry [redacted] As an example for the expected structural change, [redacted] want to investigate through immunohistochemistry and Golgi staining, the PFC and somatosensory cortical layering and axon growth ([redacted]

2. Behavior experiment

In the behavior experiment [redacted] will focus on the findings of [redacted] human data showing that the [redacted] [redacted] is associated with an increase in [redacted] function and a greater density in the [redacted] [redacted] in children with the [redacted]. This is supported by the mice data of [redacted]) as they showed a [redacted] which project toward the [redacted] Both data supports a link between [redacted] and [redacted] structure and function. Noteworthy, the [redacted] cortex is associated with autism, thereby, linking [redacted] to the development of autism.

Animal models

[redacted] model

. [redacted]

[redacted] [redacted] model:

. [redacted]

. [redacted]

Methods

Behavioral and cognitive measure studies

After [redacted] will be scored to confound for any differences in [redacted] of the [redacted] [redacted] Over time, the [redacted] [redacted] will be exposed to a variety of behavioral tasks which are related to the [redacted] [redacted] autism and require the [redacted] [redacted] to execute the tasks. Two behavioral tasks will be performed at infancy to investigate ([redacted], two other tasks will be performed at adolescence focusing on autism-related traits. After sacrifice [redacted] want to investigate brain structural/functional changes: the balance between excitatory and inhibitory neurons using fluorescence immunohistochemistry ([redacted]; [redacted]) and through immunohistochemistry and Golgi staining, the PFC and somatosensory cortical layering and axon growth ([redacted]

Go / no-go moment 1

If at least one of the pilot studies show significant differences between [redacted] a 'go' will be given, meaning that the rest of the project can start to investigate if this [redacted] is involved in [redacted]. For [redacted] a separate go / no-go will be given.

As the aim of this project is to investigate the [redacted], first [redacted] need to investigate if the [redacted] of [redacted] is visible in rats (until know only [redacted]). As [redacted] can be visible at different levels two pilot studies investigated changes at the [redacted] level, brain structural/functional level and behavior. If at one of these levels significant changes are visible a 'go' will be given.

Criteria

Significant result [redacted] experiment	Significant result [redacted] experiment	Significant result [redacted] experiment
1. Difference between [redacted] levels in the [redacted]	Difference in at least one [redacted]	Difference in at least one [redacted]
2. At least one [redacted] change in the [redacted] or [redacted]		

Study 1 (DAP 2 / 3)

In short, this study investigates in rats the effect of [redacted] and [redacted] by comparing the robust null mutant [redacted] form. The differences between these [redacted] will be studied on research question 1: [redacted] 3: ADHD/autism-related and co-morbid-related behavior and PFC and somatosensory cortical function & structure. Furthermore, with neuroimaging technologies it is possible to get a more precise and translational overview of brain structural and functional changes. Within this study two experiments with different animal procedures need to be performed, one experiment during pregnancy and one experiment throughout offspring's its life.

Experiment 1 (DAP 2)

Research questions

1. Which of the three potential [redacted] underlie the [redacted] effects?
2. How is the [redacted] by the [redacted] mediated [redacted] effects.

Animal models

Using [redacted] rat specific breeding schemes (shown in DAP 2); i.e. enabling the study of [redacted] of both [redacted] animal model

.

[redacted]

[redacted] animal model:

.

[redacted]

Methods

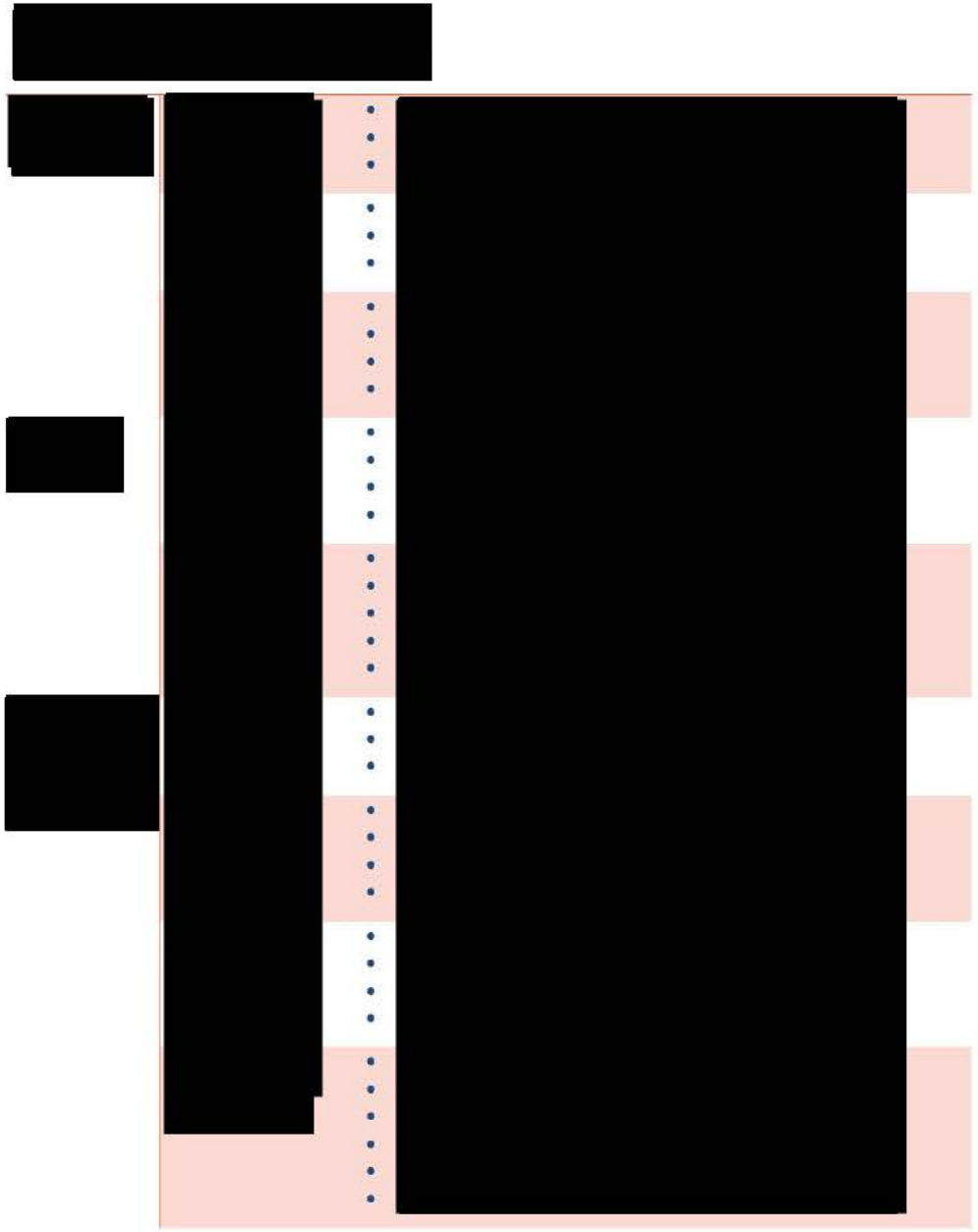
[redacted] will be divided over two groups:

1. [redacted] group ([redacted]
2. [redacted] group ([redacted]

These [redacted] will be sacrificed (decapitation or perfusion) at [redacted] to study changes within the [redacted] machinery and [redacted] related to the [redacted] from [redacted] source to [redacted]. We will collect [redacted] [redacted] and [redacted] to perform a variety of ex-vivo measurements to reveal potential mechanisms by which [redacted] and through which [redacted] changes affect risk for [redacted] disorders.

The [redacted] is studied to answer which of the three potential [redacted] is involved in ADHD/autism-related and co-morbid-related behavioral and brain structural & functional changes. For this reason [redacted] of [redacted] levels and levels of related enzymes such as [redacted]. The specific measurements are described in DAP 1 and an overview of the expected changes relating to all three pathways and their sub pathways are described in table 1. A sub pathway is influenced by the [redacted] if a max of 1 of the changes is not altered.

Table 1. [redacted] machinery changes related to the pathways and their sub pathways. These (sub) pathways are depicted in figure 2.



The [redacted] want to investigate are the balance between excitatory and inhibitory neurons using fluorescence immunohistochemistry [redacted]. As an example for the expected structural change, [redacted] want to investigate through immunohistochemistry and Golgi staining, the [redacted] and [redacted], neuronal and synaptic morphology, and formation of the [redacted].

Experiment 2 (DAP 3)

Research question

3. What are the effects of [redacted] on [redacted] of [redacted] and [redacted]

This research question can be divided into two parts:

A: [redacted] are caused by [redacted] animal model)?

B: [redacted] are caused by [redacted] animal model)?

Animal models

Using [redacted] rat specific breeding schemes (shown in DAP 1); i.e. enabling the study of [redacted] of [redacted]

[redacted] animal model

- [redacted]

[redacted] animal model:

- [redacted]

Methods

Behavioral and cognitive measure studies

After delivery, [redacted] will be scored to confound for any differences in [redacted]. Over time, the [redacted] will be exposed to a variety of behavioral tasks which are related to [redacted] and require the prefrontal and/or somatosensory cortex to execute the tasks, hence, the selected tasks are focusing on Diagnostic and Statistical Manual of Mental Disorders (fifth edition) criteria of autism, ADHD, anxiety/depression or OCD. Because [redacted] want to investigate the expected aberrant behavior of the offspring over time, these tasks will be studied longitudinally: [redacted]. At every time period, the test battery will start with the shortest and least stressful task and will end with the most stressful task. This is done to diminish the influence of the already-performed-tasks. Furthermore, tasks which are repeated at different time points will be performed under different environments to ensure the novelty of the environment and thereby to avoid potential carry-over effects. The (order of the) tasks will be explained in more detail in research outline 3.4.2. and in DAP 3.

Given the amount, duration and certain learning-dependency of the behavioral tasks and the short-time period to study a certain [redacted] stage (i.e. [redacted]) it is crucial to divide the behavioral tasks over four groups:

- [redacted] group
- [redacted] group
- [redacted] group
- [redacted] group A & B

After the last behavioral tasks at adulthood, the animals will be sacrificed (decapitation or perfusion) to study functional and structural changes in the brain. The [redacted] want to investigate are in line with the focus of the functional and structural analyses performed under research question 2: the [redacted],
[redacted]).
As the division of the five groups is based on disease-related Diagnostic and Statistical Manual of Mental Disorders (fifth edition) criteria, changes in PFC / somatosensory cortex structure and function can be interpret towards disease-related traits as well.

Brain structure & function studies
Besides the behavioral groups another group of animals will be exposed to an MRI scanner at , and to non-invasively study structural and functional changes.

- [redacted] group

Naïve groups are added as well to study brain structural and functional changes throughout development without an influence of behavior.

- Naïve group for [redacted]
- Naïve group for [redacted]
- Naïve group for [redacted]

Go / No-go moment 2
In the first study, the robust animal models are used (i.e. [redacted]). If the results show significant differences between [redacted] and/or [redacted] in comparison to [redacted] rats, [redacted] will proceed with investigating [redacted] rats (go / no-go moment). The definition of a significant main effect is explained in the statistics of DAP 2: A.3.

Study 2 (DAP 2 / 3)
If study 1 supports a clear influence of the [redacted] effect, a second study starts investigating whether these effects are comparable to [redacted] in comparison to [redacted] For this reason, the design of study 2 is exactly the same as study 1 (DAP 2/3) with the exception of the animal models: [redacted] will be used instead of [redacted]. Noteworthy, only if [redacted]g is used in study 1 the [redacted] needs to be investigated for [redacted] before study 1 can be replicated; this is described in DAP 3.

- [redacted]
- [redacted]

Reference

Bengel, D., Murphy, D. L., Andrews, A. M., Wichems, C. H., Feltner, D., Science, C., & Maryland, D. B. (1998). Altered Brain Serotonin Homeostasis and Locomotor Insensitivity to 3 , 4-Methylenedioxymethamphetamine (" Ecstasy ") in Serotonin Transporter-Deficient Mice. Molecular Pharmacology, 53, 649–655. doi:10.1124/MOL.53.4.649

[redacted]

3.4.2 Provide a basic outline of the different components of the project and the type(s) of animal procedures that will be performed.

Experiment 0. (DAP1) Pilot studies
Behavioral and cognitive measure studies
The first animal procedure is measuring behavior. Four behavioral tasks are chosen related to autism-traits as [redacted] has already shown that in all tasks significant differences were found in behavior between [redacted] rats and [redacted] and vehicle-exposed Wistar rats.

[redacted]

[redacted]

The second type of animal procedure is sacrifice by decapitation to collect [redacted] rats or by decapitation or perfusion to collect adult brains after behavior.

[redacted] levels

To assess if [redacted] is measured using HPLC in [redacted]

[redacted] assays during [redacted] period and adulthood

Balance between inhibitory and excitatory signals in PFC

To assess if [redacted] influences the balance between inhibitory and excitatory signals coronal sections are double stained for NeuN (all neurons) and GAD67 (glutamic acid decarboxylase 67, marks GABA-ergic neurons). NeuN+ and GABA+ neurons are inhibitory, NeuN+ and GABA- neurons non inhibitory, probably excitatory.
[redacted]
Coronal sections are stained for [redacted] and Satb2 (special AT-rich sequence-binding protein 2); satb2+ as a marker for callosal projection neurons in cortical layers II-VI. This measurement will be performed as described by [redacted]
Axon growth & dendritic pruning
To assess axon growth and dendritic pruning in the forebrain Golgi staining will be used. This measurement will be performed as described by [redacted]

Experiment 1. (DAP2) Identification of [redacted] underlying the [redacted] actions of [redacted]
The ***main type* of animal procedure** is sacrifice by decapitation or perfusion to collect [redacted]t rats.
Balance between inhibitory and excitatory signals in PFC
To assess if [redacted] influences the balance between inhibitory and excitatory signals coronal sections are double stained for NeuN (all neurons) and GAD67 (glutamic acid decarboxylase 67, marks GABA-ergic neurons). NeuN+ and GABA+ neurons are inhibitory, NeuN+ and GABA- neurons non inhibitory, probably excitatory.
Cortical neurogenesis activity
To assess if [redacted] influences cortical neurogenesis activity a variety of staining's are used, for proliferation Ki67 and BrdU; for differentiation DCX; stemness Sox2 and Nestin; cell death CC3; neurons Map2 and astrocytes GFAP.
[redacted]
To assess if [redacted] influences the formation of the [redacted] network coronal sections are stained for [redacted] and Satb2 (special AT-rich sequence-binding protein 2); satb2+ as a marker for colossal projection neurons in cortical layers II-VI.
[redacted]
To assess if [redacted] influences neuronal and glial development axons and dendrites are stained using Golgi staining.

Experiment 2. (DAP3) Identification of psychiatric disease-related behavior & brain functional and structural traits
[redacted] *studies*
The **<u>first type of animal procedure</u>** is the scoring of [redacted] in the [redacted], mainly focusing on three ways of [redacted].

[redacted] *studies*
The **<u>second type of animal procedure</u>** female rats with [redacted]

Behavioral and cognitive measure studies
<u>The third animal procedure</u> is measuring behavior during a variety of disease-related task focusing on ADHD, autism, anxiety, depression and OCD.
1. *<u>Autism-related traits group</u>*

Autism-related behavioral traits including: 1. social interaction (social play); 2. social communication (olfactory habituation/dishabituation to odors); 3. stereotyped/repetitive motor movements (self-grooming behavior); 4. hyper- or hypo-reactivity to sensory input (prepulse inhibition and robotic gap crossing task); 5. insistence on sameness/routines (reversal learning / novel object recognition); 6. highly abnormal focus & fixated interests in unusual objects / restricted interest (object directory behavior); 7.control for reflex development (negative geotaxis).

2. *ADHD-related traits group*

ADHD-related behavior traits including: 1. hyperactivity (open field test); 2. inattention (Y-maze or T-maze with four closed arms) and 3. impulsivity (five serial choice task using a touch screen).

3. *Co-morbid anxiety / depression-related traits groups group*

Anxiety-related behavior traits through the elevated plus maze and depression-related behavior traits including: 1. pessimism (ambiguous cue interpretation test), 2. psychomotor retardation (forced swim test) and 3. markedly diminished interest or pleasure in activities (sucrose preference test).

4. *Co-morbid OCD-related traits group A & B*

Obsessive-compulsive disorder-related traits including: 1. sensory compulsivity (schedule-induced polydipsia procedure) and motor compulsivity (signal attenuation task).

Brain structure & function studies

The fourth type of animal procedure is measuring non-invasively structural and functional changes over time.

Neuroimaging for brain structure & function:

Neuroimaging tasks including: sMRI (structural data), fMRI (functional data), diffusion tensor imaging (DTI for white matter tractography), arterial spin labeling of PFC and somatosensory cortex (ASL for functional data). Noteworthy, due to these neuroimaging techniques it will be possible to measure [redacted].

The fifth type of animal procedure is sacrifice by decapitation or perfusion. In this way, the brains can be used to study brain structure and function by performing ex-vivo tests. Beside the 4 groups described above the tree naïve group described in the research strategy are studied as well as well to investigate brain structural and functional changes throughout development without an influence of behavior.

Balance between inhibitory and excitatory signals in PFC

To assess if [redacted] influences the balance between inhibitory and excitatory signals coronal sections are double stained for NeuN (all neurons) and GAD67 (glutamic acid decarboxylase 67, marks GABA-ergic neurons). NeuN+ and GABA+ neurons are inhibitory, NeuN+ and GABA- neurons non inhibitory, probably excitatory.

Hippocampal neurogenesis activity

To assess if [redacted] influences hippocampal neurogenesis activity a variety of stainings are used, for proliferation Ki67 and BrdU; for differentiation DCX; stemness Sox2 and Nestin; cell death CC3; neurons Map2 and astrocytes GFAP.

Formation of the [redacted]

To assess if [redacted] influences the formation of the [redacted] network coronal sections are stained for [redacted] and Satb2 (special AT-rich sequence-binding protein 2); satb2+ as a marker for colossal projection neurons in cortical layers II-VI.

Reference

[redacted]

[redacted]

3.4.3 Describe the coherence between the different components and the different steps of the project. If applicable, describe the milestones and selection points

Coherence

As shown in figure 4 this project is divided in two studies representing two different animal models for mimicking human [redacted] (study 1 [redacted]) and human [redacted] (study 1 [redacted] vs. study 2 [redacted] Both studies can be sub-divided in two experiments (DAP 2 and DAP 3). In the first experiment [redacted] study the effect of [redacted] on the [redacted] (R. Q. 1 & 2). In the second experiment we study the effect of [redacted] genotype on behavior and brain structure and function in relation to [redacted] disorders and their co-morbid diseases.
This strategy allows us to study how the [redacted] influences the [redacted] machinery, how this affect influences [redacted] and if these [redacted] can lead to [redacted] disorders, ADHD and/or autism, and/or co-morbid disorders such as anxiety, depression and OCD.

Go / no-go moment 1

Before the start of study 1 a go / no-go moment is implemented to investigate if the [redacted] hypothesis also holds true in our rat models. As shown in mice and human data [redacted] can result in altered [redacted] levels, changes in [redacted] changes in brain structure/function and eventually changes in behavior. For this reason, two pilot studies will investigate changes at the [redacted] level, brain structural/functional level and behavior. If at one of these levels significant changes are visible a 'go' will be given. After the 'go' the rest of the project can start to investigate if this [redacted].

Go / no-go moment 2

Between study 1 and 2 a go / no-go moment is implemented. The second study will be performed with the superior animal model; looking from a genetic point of view. This study will only start if there are significant main effects showing that the [redacted] model results in ADHD/autism-related or co-morbid-related behavior and brain structural/functional traits which are related to at least one of the [redacted] and caused by [redacted] changes. The definition of a significant main effect is explained in the statistics of DAP 3: A.3. The second study is only performed with the gender where the significant results were found.

3.4.4 List the different types of animal procedures. Use a different appendix 'description animal procedures' for each type of animal procedure.

Serial number	Type of animal procedure
1	Pilot study
2	Identification of [redacted] and its [redacted] underlying the [redacted]
3	Identification of [redacted]

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 1	Type of animal procedure ■ study

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

General design & Primary outcome measures

As the evidence for the [redacted] comes from human and mice data, first two pilot studies will be performed to investigate if the [redacted] is also seen in these rat models. If at least one of the pilot studies show significant differences between [redacted] of [redacted] a 'go' will be given, meaning that the rest of the project can start.

[redacted] study: [redacted] will be sacrificed at [redacted]). Rats will be sacrificed using rapid decapitation without anesthesia; for histological read-outs [redacted] brains will be fixed overnight with a fixative and for molecular read-outs fresh brains will be frozen.

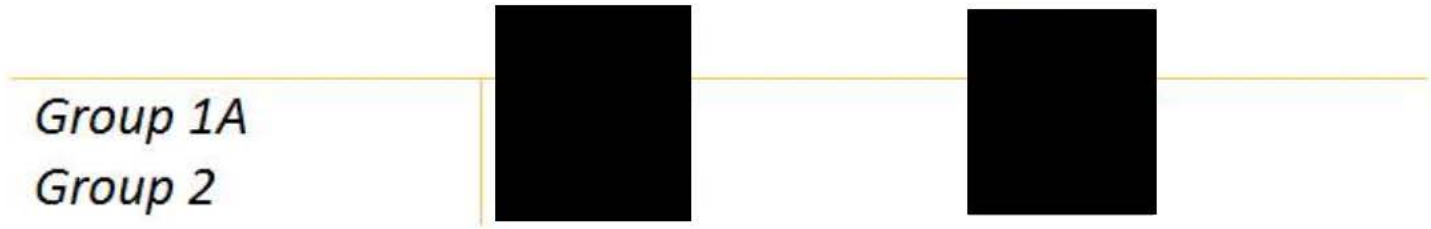
Autism-related traits behavior study: [redacted] will be exposed to a variety of autistic-related tasks at infancy and adulthood. Directly afther the last behavior rats will be sacrificed by perfusion or decapitation and their brains analyzed for changes in brain structure and function.

Justification:

This experiment investigates if [redacted] is needed and if the [redacted] is also seen in these rat models.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

[redacted] ***of animals***
[redacted] **study**



study

METHOD of PILOT study

To reveal the potential mechanisms (figure 2) by which rats of batch A ( ) and B (genotype at when the is fully dependent on (figure 1 & 2).

Sacrifice

rats and their will be decapitated to collect trunk blood of .

- and is needed for HPLC to investigate levels.

- [redacted] as fixed (brain) material is needed for immunohistochemistry to investigate [redacted].
[redacted] *changes*
[redacted] levels will be measured in [redacted].
[redacted] *assays*
- To assess [redacted] inhibitory and excitatory signals in terms of PFC and somatosensory cortical structure. Coronal sections are double stained for NeuN (all neurons) and GAD67 (glutamic acid decarboxylase 67, marks GABA-ergic neurons). NeuN+ and GABA+ neurons are inhibitory, NeuN+ and GABA- neurons, non-inhibitory, probably excitatory.
- To assess the formation of the [redacted]. Coronal sections are stained for [redacted] and Satb2 (special AT-rich sequence-binding protein 2); satb2+ as a marker for [redacted] in [redacted]. This measurement will be performed as described by [redacted]
- To assess axon growth and dendritic pruning in the [redacted] golgi staining will be used. This measurement will be performed as described by [redacted]

METHOD of PILOT behavior study

Behavioral and cognitive measure studies

The offspring of batch C ([redacted]) and D ([redacted]) will undergo a variety of (PFC or somatosensory cortex-dependent) behavioral tests; shown in figure 6. Four behavioral tasks are chosen related to autism-traits as [redacted] group has already shown that in all tasks significant differences were found in behavior between [redacted] rats and between [redacted] and vehicle-exposed Wistar rats ([redacted]).

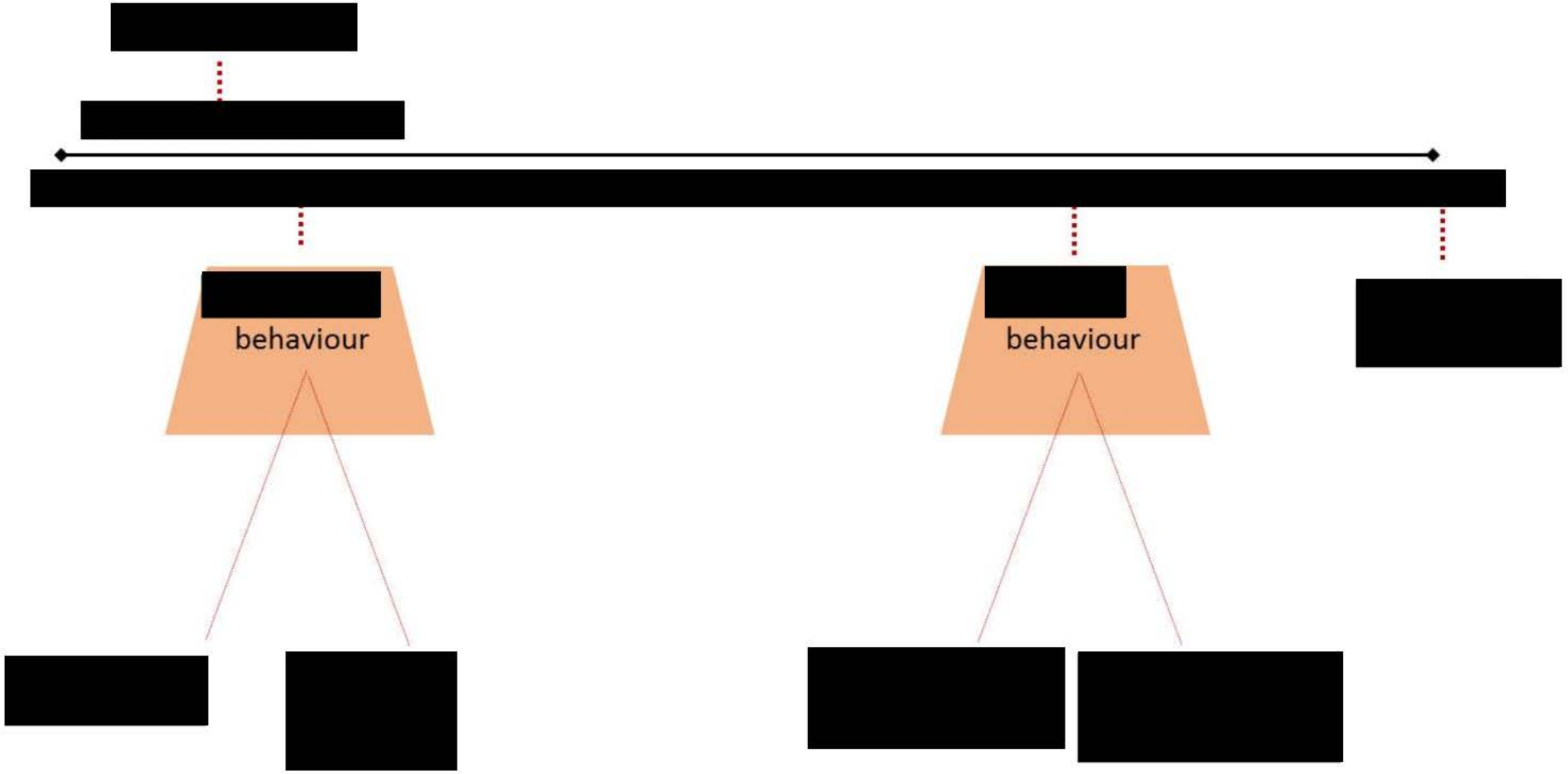


Figure 6. Timeline animal study. [redacted] rats are exposed to a test battery at early life behavior and adolescence behavior.

Negative geotaxis

The negative geotaxis is used as a [redacted] and is studied between [redacted] The pup will be placed on a tilted surface (40°) with its head facing downwards and the time period until turning 180° or walking upwards is scored.

Olfactory discrimination

Olfactory discrimination is used to investigate social olfactory function (social communication). The pup will be placed in the center of an empty cage in a neutral position. On one side of the cage, fresh sawdust will be placed, while on the other side bedding from the homecage (including several pieces of faces) will be placed. The side of the fresh sawdust is changed every day, to prevent bias caused by environmental cues. The time the rat needs to reach the bedding from its homecage (with their mothers odor) will be measured. The experiment ends when the rat reached the bedding from its homecage or after 240 s. Olfactory discrimination will be measured at [redacted]

Social play
Social play studies the interaction between rats which can be used to social interaction deficits. This test relies upon the fact that rats at their adolescence age show high social play behavior, animals showing autism-related treats will play less. Two days before the experiment the rat will already be placed for 10 minutes in the plastic cage (40×40×60 cm [l×w×h]) with approximately 2 cm of wood shavings covering the floor to habituate.
On the test day, test pairs are isolated for a maximum of 24h before the test to induce a half-maximal increase in the amount of social play behavior (Niesink & Van Ree, 1989). Two rats of similar gender, weight (difference < 10 g) and [redacted] breed and with no social contact before will be put with each other in the cage for 15 minutes. Social interaction of rats will be measured at [redacted]
Multiple behaviors will be scored, including:

- pinning: one of the animals lying with its dorsal surface on the floor of the test cage with the other animal standing over it
- pouncing: play soliciting by nosing the partner's nape
- boxing/wrestling: facing each other in vertical position and struggling using the forepaws
- following/chasing: moving in the direction of or pursuing the test partner, who moves away
- social grooming: licking any body part of test partner
- social exploration: sniffing any body part of the test partner

Robotic (spontaneous) Gap crossing task
Robotic gap crossing is used to study the somatosensory (whisker) function (Pang et al., 2011) of an animal which is often altered in autism. Animals are placed on one of the two elevated platforms separated from each other with randomly varying gap-distance (range: 3–8 cm, step-size: 0.5 cm) and their probability of successful object localization across gap-distances is quantified. The training is performed under infrared light and white noise. Animal mobility on the platforms is quantified using custom-made infrared motion sensors placed at the two ends and the middle of each platform. Trial duration, duration of sensory exploration at the gap, number of attempts prior to successful gap crossing, and duration of mobility are scored (Pang et al., 2011). Somatosensory (whisker) function is measured at time point [redacted]

Ex vivo: Prefrontal and somatosensory cortical architecture
After the experiments male and female offspring are perfused with 4% paraformaldehyde or decapitated (figure 6 – 8 and 10, 11) to investigate brain function and structure through a variety of measurements. The ex-vivo measurements are described in the next session 'Brain structure & function studies'
Balance between inhibitory and excitatory signals in PFC
Coronal sections are double stained for NeuN (all neurons) and GAD67 (glutamic acid decarboxylase 67, marks GABA-ergic neurons). NeuN+ and GABA+ neurons are inhibitory, NeuN+ and GABA- neurons non inhibitory, probably excitatory.
Somatosensory cortex layering & *Formation of the* [redacted]

Coronal sections are stained for [redacted] and Satb2 (special AT-rich sequence-binding protein 2); satb2+ as a marker for colossal projection neurons in cortical layers II-VI.

References

[redacted]
Niesink, R. J. M., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. Neuropharmacology, 28(4), 411–418. http://doi.org/10.1016/0028-3908(89)90038-5
Pang, R. D., Wang, Z., Klosinski, L. P., Guo, Y., Herman, D. H., Celikel, T., … Holschneider, D. P. (2011). Mapping functional brain activation using [14C]-iodoantipyrine in male serotonin transporter knock-out mice. PLoS ONE, 6(8), e23869. http://doi.org/10.1371/journal.pone.0023869
[redacted]

Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Throughout all procedures data dropout and loss of animals will be minimized by careful execution of the experiments and close monitoring animal welfare. For the behavior [redacted] estimate that [redacted] need maximally 15 male rats per group; based on previous work [redacted] for [redacted] study [redacted] need 4 dams to investigate [redacted] levels and [redacted] assays; based on previous work [redacted] will calculate the precise group sizes per experiment using a power analysis (alfa 0.05), based on data collected so far by us and others.

References

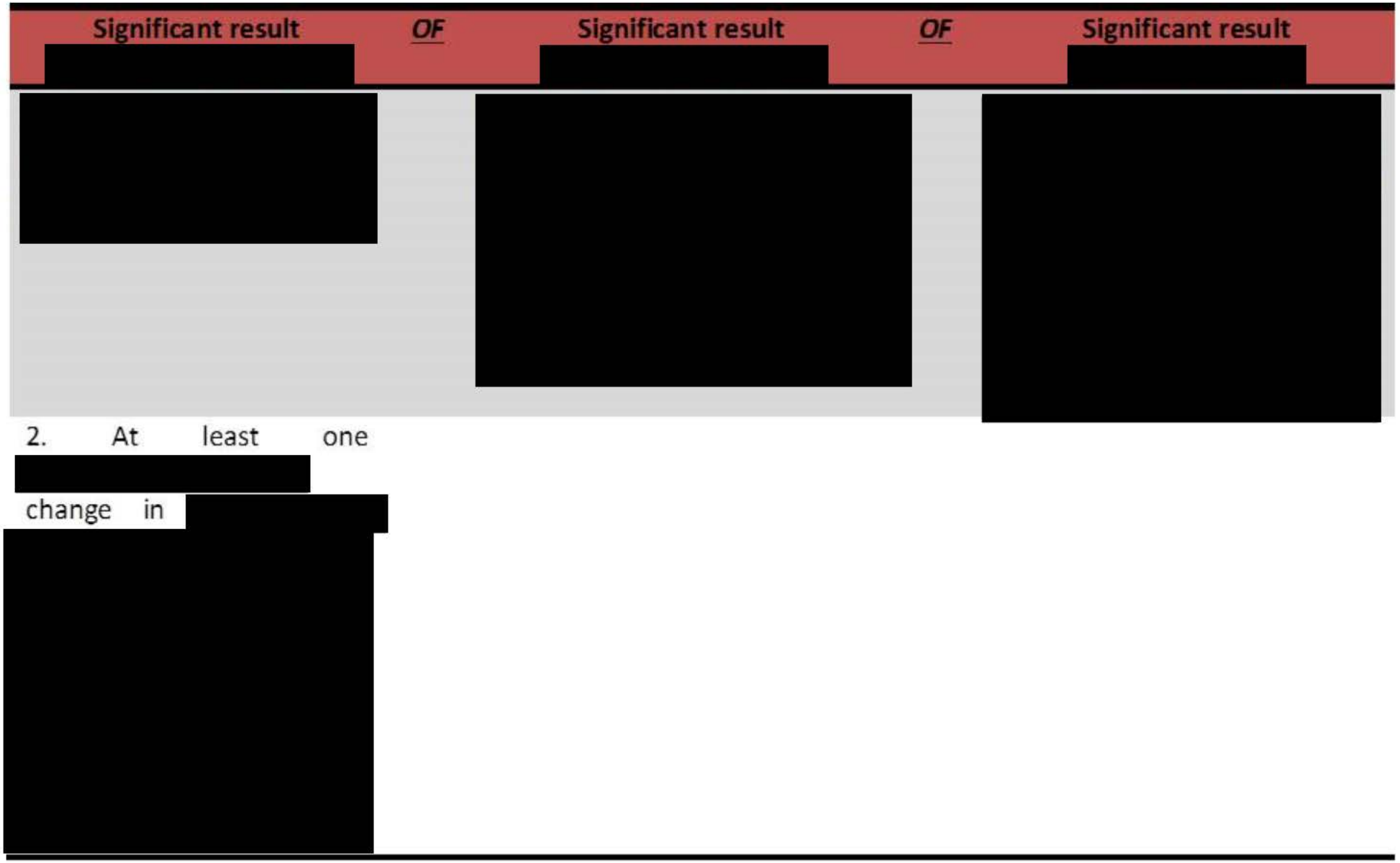
[redacted].

Go / no-go moment 1: analysis pilot studies

To investigate if a go or a no-go needs to be given two pilot studies are performed; one focusing on the earlier published [redacted] data of [redacted]., [redacted] and one focusing on [redacted]. [redacted] actions can result

in altered [redacted] levels, changes in [redacted], changes in brain structure/function and eventually changes in behavior. If at one of these levels significant changes are visible a ‘go’ will be given. After the ‘go’ ‘study 1’ can start to investigate if this [redacted] hypothesis is involved in [redacted]

Criteria


Significant result
OF
Significant result
OF
Significant result
2. At least one
change in

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

[redacted] use the [redacted]) and [redacted] and [redacted], respectively. [redacted] use these [redacted] rats as [redacted] models for [redacted] and [redacted] [redacted] rat line is [redacted] by [redacted] [redacted] at the [redacted] The [redacted] rats are provided by [redacted], however, further breeding towards [redacted] offspring will be also by [redacted]. Both [redacted] are major regulators of [redacted]. Changes in [redacted] can affect [redacted] [redacted] in blood and/or [redacted]). Since the [redacted] during the first half of [redacted] these peripheral processes have the potency to alter the [redacted].
Because this is a pilot study focusing on autistic-related traits only males will be used.
The breeding of [redacted] animals is a breed without any discomfort. The breeding of [redacted] animals is however probably a breed with discomfort as in mice [redacted]).

Table 2. Animal batches per pilot experiment

Study	[redacted] *animal model*	[redacted] *animal model*
[redacted]	[redacted]	[redacted]
[redacted] *study*		
[redacted] *study*		

The following groups are proposed:
PILOT [redacted] STUDY
[redacted]
1. [redacted] group (Batch A); TOTAL: 4 animals
2. [redacted] group (Batch A); TOTAL: 4 animals
[redacted]
1. [redacted] group (Batch B); TOTAL: 4 animals
2. [redacted] group (Batch B); TOTAL: 4 animals
[redacted]
1. [redacted] therefore, males need to be counted as well:
- [redacted] group (Batch B); TOTAL: 4 animals
- [redacted] group (Batch B); TOTAL: 4 animals
TOTAL PILOT STUDY: 24

PILOT BEHAVIOR STUDY
[redacted]
1. [redacted] animals from [redacted], n=15 per group (Batch C),
however when [redacted]: n=30 (n=15 per gender for [redacted] of [redacted]r and n=15 per gender for [redacted]
[redacted]
2. [redacted] animals from [redacted], n=15 per group (Batch C),
however when [redacted]: n=30 (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted]
[redacted]
1. [redacted] animals from [redacted] n=15 per group (Batch D),
however when [redacted]: n=30 (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]
[redacted]
2. [redacted] animals from [redacted], n=15 per group (Batch D),
however when [redacted]: n=30 (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]
[redacted]
1. [redacted]
è A total of 60 [redacted] is needed (Batch D)
è [redacted] needed. Total of 20 animals.
TOTAL FOR BEHAVIOR EXPERIMENT: 140 ANIMALS (without [redacted]: 70)

TOTAL all rats: 164 animals

References

[redacted]

Species	Origin	Maximum number of animals	Life stage
Rat	[redacted]	8	[redacted]
Rat		60	
Rat		36	
Rat		60	

C. Re-use

Will the animals be re-used?

[] No, continue with question D.
[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Adult breeding females and males can be used for another breeding if their health and age allow it, so these animals can go back to the breeding WP.

Are the previous or proposed animal procedures classified as 'severe'?

[X] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
The rat is the best animal model to perform behavioral studies to investigate [redacted] and [redacted] disorders. Because of the complexity of these kind of disorders, it is impossible to use lower-order animals. Furthermore, since it is a [redacted] we can study the onset and development of disease traits from an early age on. Specific (brain) mechanisms cannot be studied in vivo in humans, because of ethical restraints. Therefore, it is of importance to use rats to investigate these routes.

Reduction
The requested amount of animals (based on a group size of n = 4 and 15) is needed for statistical reliable conclusions and is the minimal group size one can work with. Moreover, the same animals will be used for a variety of ex vivo studies to obtain a high number of information thereby leading to a minimal amount of animals needed.

Refinement
The experiments will be carried out with the least discomfort possible. For this reason, social housing before and during [redacted] and cage enrichment will be applied. Moreover, the analysis [redacted] propose cannot be performed without sacrificing animals. As usual, all efforts will be undertaken to minimize animal suffering during sacrifice. Only experienced researchers will handle the animals.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

[redacted] will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked twice a week to be able to detect Human End Point conditions. The animals will be weighted only during the cleaning of the cages, in this way, handling of the animals will be minimized to reduce human intervention that may cause stress for the animals. Decapitation will be performed by experienced researchers.
The discomfort [redacted] rats will be exposed to is limited to the absolute minimum necessary [redacted]. Animals will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked on a daily basis to be able to detect Human End Point conditions. At a young age the animals will be weighted daily which will decline to once a week from adolescent age onwards. Handling of the animals will be minimized to reduce human intervention that may cause stress for the animals. Perfusion will take place under deep anesthesia to minimize adverse effects.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

[] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

Some of the behavioral tests can cause stress at the psychological level. For instance, social interaction as the animals need to be single housed for 24 hours before the start of the experiment.

Explain why these effects may emerge.

The animals need to be single housed for 24 hours to increase social interaction.
Because the decapitation without anesthesia will be done by an experienced experimenter, and will take place in a fraction of a second, no pain or adverse effects are expected from the decapitation. As the fetuses of the dams will be decapitated directly after the decapitation of the dam their discomfort will be minimized.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Although some stressors are inherent to the experimental design, [redacted] will take precautionary measures to minimize all other potential causes of (additional) stress to the animals, e.g., by socially housing the rats with cage enrichment and only partial cleaning of the housing cages to retain hierarchy (and thereby prevent fighting to re-establish this hierarchy).

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

The criteria to take the animal out of the experiments are based on human observation of factors known as clear symptoms of pain/stress/discomfort and defined for humane end point detection*. Weight loss of more than 15% in one/two days and 20% over the whole study are considered as humane endpoints. Also (a combination of) general symptoms such as raised fur, hunched back (arched back), poor coat conditions, are also considered as humane endpoints after which the animals should be euthanized.
For [redacted] rats an additional humane endpoint will be applied concerning their [redacted] [redacted] a combination of clear symptoms of breathing difficulties, progressive pallor and signs of fatigue.
*Standard humane endpoints rodents: piloerection, loss of body weight (> 15%), immobility, poor self-care, tremor, self-damage, abnormal body posture, convulsions, tumors, elephant teeth.

Indicate the likely incidence.

As [redacted] is a breeding without discomfort it is unexpected that any of the pregnant dams reach the human end point over the course of the experiment (< 0%).
It is unexpected that any of the [redacted] (breeding with discomfort) and behavioral animals reach the human end point over the course of the experiment (< 2%).

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The total (cumulative) discomfort of the [redacted], all the dams and the behavioral animals are expected to be mild.
All [redacted] and dams are not exposed to behavioral challenges and therefore they will not experience any suffering. Furthermore, as the behavioral tasks only include small stressors (i.e. 24h single housing) the behavioral animals will be exposed to a mild discomfort.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

The [redacted] of the [redacted] are needed to analyze the brain structure and function in the brains of the fetus investigating changes underlying ADHD- and autism-related and co-morbid disease-related traits. Further, the brains of the behavioral animals are needed to analyze the brain structure and function investigating changes underlying ADHD- and autism-related and co-morbid disease-related traits.
Except the [redacted] breeding males and females. These rats can go to the breeding protocol.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 2	Type of animal procedure Identification of [redacted] and its [redacted] underlying the [redacted]

2 Description of animal procedures

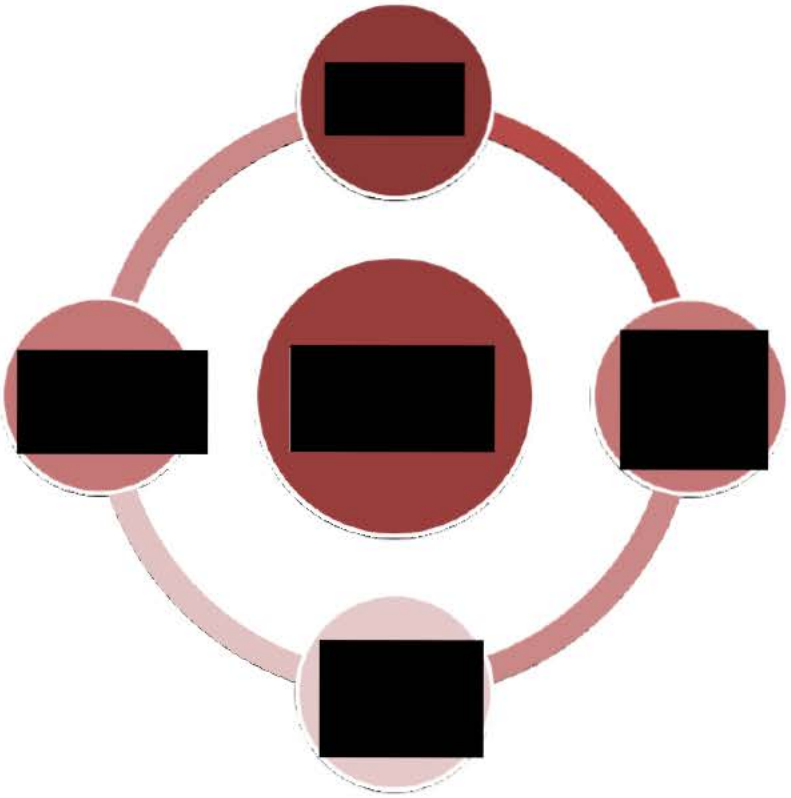
A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

General design & Primary outcome measures

With the experiments in this DAP 2 [redacted] to determine [redacted] underlying ADHD/autism-related and co-morbid-related behavioral and brain structural and functional traits in offspring of [redacted] (study 1) or [redacted] (study 2) mothers, by 1) investigating changes at the molecular level of three potential [redacted] and 2) investigating changes in [redacted] (figure 7).

In this part [redacted] or at [redacted] Rats will be sacrificed using rapid decapitation without anesthesia; for histological read-outs [redacted] brains will be fixed overnight with a fixative and for molecular read-outs fresh brains will be frozen.



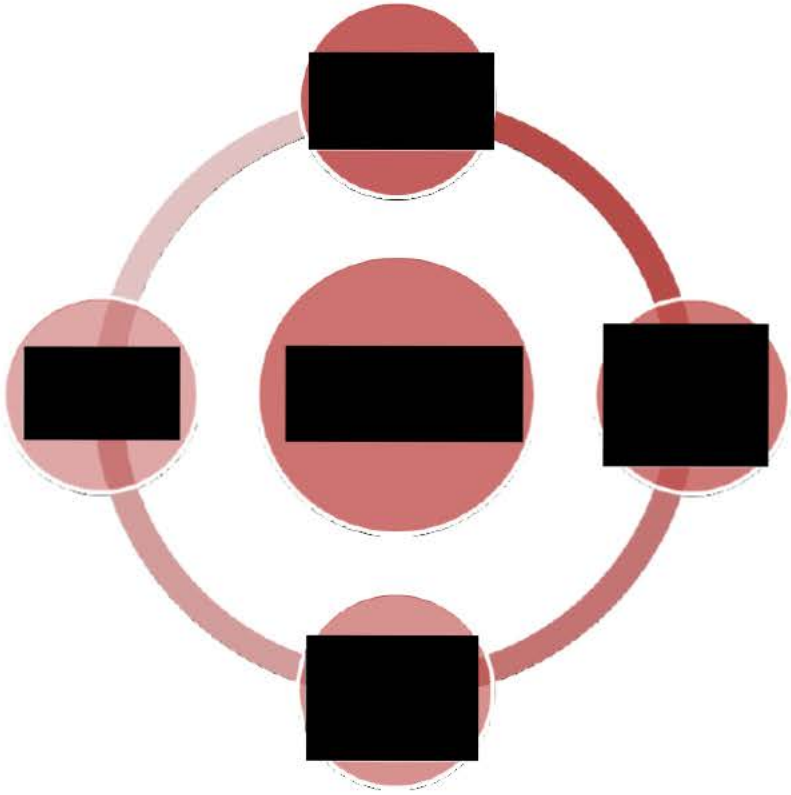
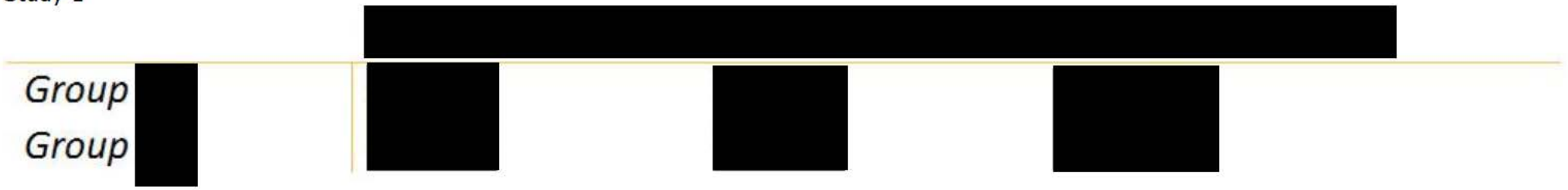


Figure 7. Research design and primary outcomes of animal procedure 2. **Left on top** experimental aim; **right on top** primary outcome measures [redacted] concerning the [redacted]; **Left bottom** primary outcome measures ex-vivo tests for [redacted] changes.

Justification:
This experiment investigates the mechanisms underlying the effects of [redacted] on [redacted] in the offspring. This is (ethically) not possible in humans but necessary to understand in trying to prevent/treat such a disorder.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

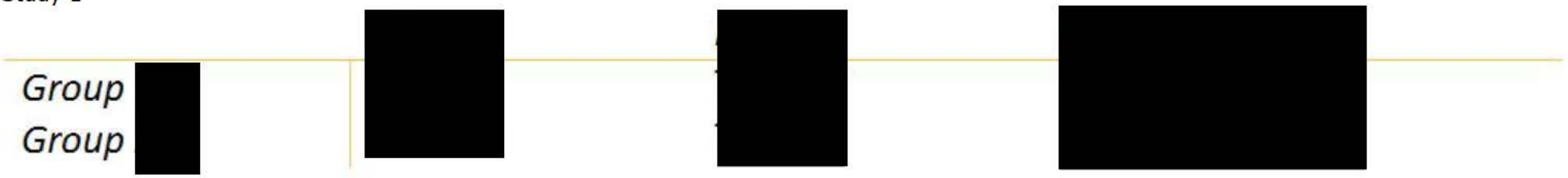
[redacted] ***of animals***
Note that in both cases offspring all have the same [redacted] differs in the three groups.
Genotype [redacted] *animals*
Study 1



Study 2

[redacted] *animals*

Study 1



Study 2

measures

group (

To reveal the potential mechanisms (figure 2) by which and of pregnant rats of batch 13 (animals) and 14 (animals) at when the is fully on (figure 1 & 2).

group (

To reveal the potential mechanisms (figure 2) by which and of pregnant rats of batch animals) and 16 (animals) at when the is on (figure 1 & 2).

Sacrifice

Pregnant rats and their fetuses will be decapitated to collect trunk

- Half of the brains will directly be frozen as fresh (brain) is needed for HPLC/qPCR/western blot measurements.
- Half of the brains will be fixed overnight in 4% paraformaldehyde as fixed (brain) material is needed for immunohistochemistry.

As shown in table 1, PP 3.4.1 changes in the [redacted] needs to be investigated to understand which ([redacted] underlies [redacted] pathway. The needed measurements are described below:

HPLC (High-performance liquid chromatography)
HPLC will be performed as described in an earlier paper from this group: [redacted]).
[redacted] measuring tissue [redacted] levels.
[redacted]: measuring [redacted] levels.
[redacted] measuring [redacted] levels in [redacted] and [redacted]

RT-qPCR (Real time quantitative polymerase chain reaction)
[redacted]: measuring mRNA (gene) expression levels of the [redacted].

Western blot
[redacted]: measuring protein expression levels of the [redacted]

Immunohistochemistry
Immunohistochemistry will be performed as described in an earlier paper from this group: [redacted]).
[redacted] measuring [redacted] expression levels. This staining will provide us histological information. Additional immunostainings will be conducted based on new literature findings.

For the measurement of [redacted]), a variety of experiments will be performed in the [redacted] brain using immunohistochemistry:
- To assess offspring's inhibitory and excitatory signals in terms of PFC and somatosensory cortical structure. Coronal sections are double stained for NeuN (all neurons) and GAD67 (glutamic acid decarboxylase 67, marks GABA-ergic neurons). NeuN+ and GABA+ neurons are inhibitory, NeuN+ and GABA- neurons, non-inhibitory, probably excitatory.
- To assess offspring's neurogenesis/embryogenesis and cell migration in the forebrain, a variety of stainings can be used, for proliferation Ki67 and BrdU; for differentiation DCX; stemness Sox2 and Nestin; cell death CC3; neurons Map2 and astrocytes GFAP.
- To assess the formation of the [redacted] Coronal sections are stained for [redacted] as a marker for callosal projection neurons in cortical layers II-VI. This measurement will be performed as described by [redacted]).
- To assess axon growth and dendritic pruning in the forebrain golgi staining will be used.

References

[redacted]

[redacted]

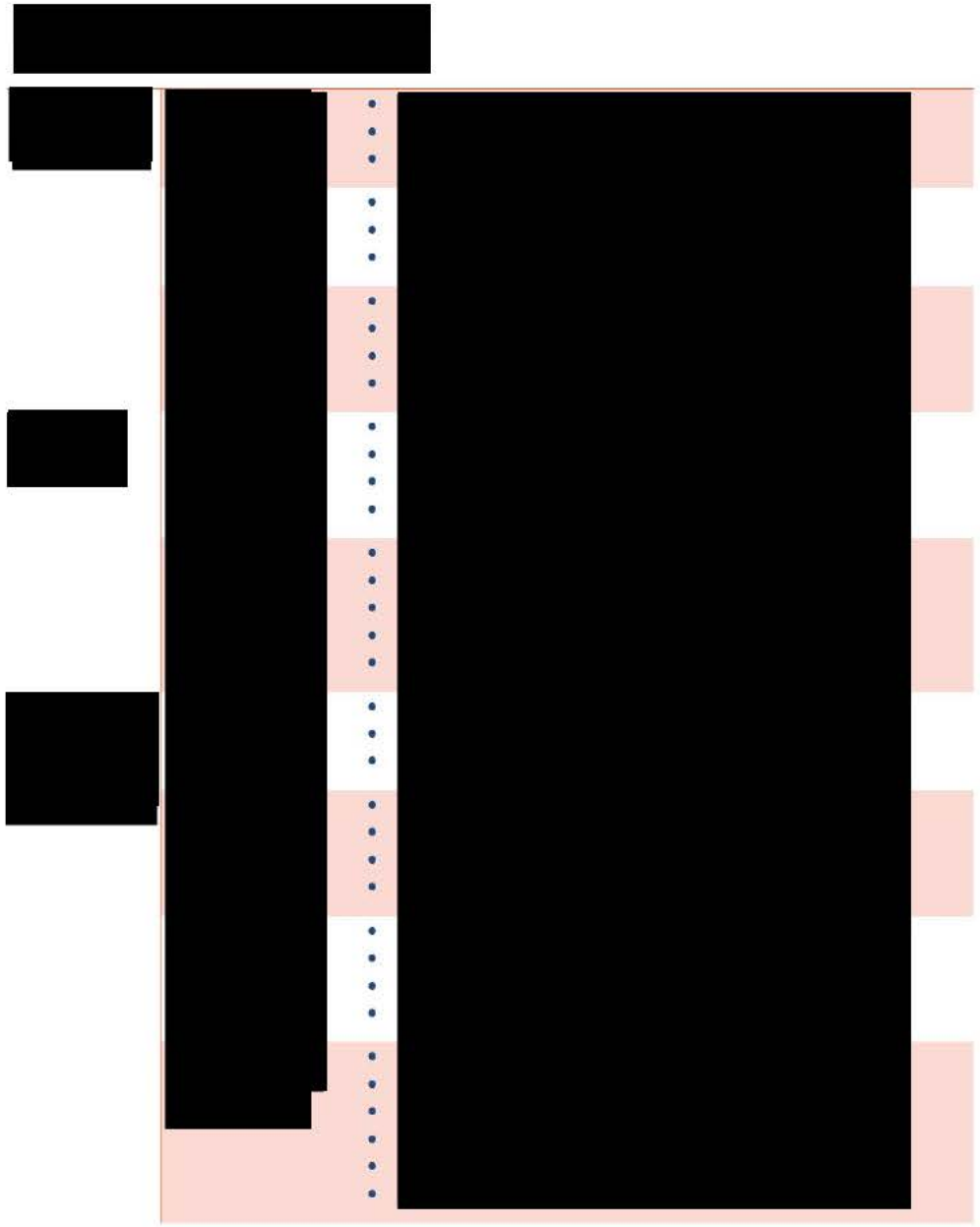
Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Throughout all procedures data dropout and loss of animals will be minimized by careful execution of the experiments and close monitoring animal welfare. [redacted] estimate that we need maximally 7 [redacted] per group when all [redacted] have the [redacted]; based on previous work ([redacted]). [redacted] will calculate the precise group sizes per experiment using a power analysis (alfa 0.05), based on data collected so far by us and others

[redacted]

Go / no-go moment 2: between [redacted] dams & analyses study 1 & 2
Only the investigated [redacted] which show significant differences between pups of [redacted] will be studied in 'study 2'.
Table 2. [redacted] changes related to the [redacted]. These (sub) pathways are depicted in figure 2 in the project proposal.

[redacted]



B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

[redacted] use the [redacted]) and [redacted] rats to model the [redacted] and [redacted], respectively. [redacted] use [redacted] rats as [redacted] investigating ADHD and autism traits (see background information). The [redacted] rat line is bred by [redacted]. The [redacted] rats are provided by a company, however, further breeding towards [redacted] will be also by [redacted]. Both [redacted] are major regulators of the [redacted]. Changes in their function can affect [redacted] in blood and/or [redacted] Since the [redacted] during the [redacted] of [redacted] these peripheral processes have the potency to alter the fetal supply of [redacted]. Because gender differences play a role in the [redacted]. The breeding of [redacted] animals is a breed without any discomfort. The breeding of [redacted] animals is however probably a breed with discomfort as in mice the null mutants from [redacted] crosses [redacted]
The following groups are proposed:

STUDY 1

[redacted]

1. [redacted], n=7 per group [redacted] animals = 14 animals
2. [redacted], n=7 per group [redacted] animals = 14 animals

TOTAL [redacted]: 28 ANIMALS

[redacted]

3. [redacted] = 42 [redacted]

4. [redacted] = 42 [redacted]

TOTAL FOR [redacted]: 84 ANIMALS

[redacted]

5. [redacted] animals = 14 animals
6. [redacted] animals = 14 animals

TOTAL FOR [redacted] 1 dams: 28 ANIMALS

[redacted]

7. [redacted] = 42 [redacted]

8. [redacted] = 42 [redacted]

TOTAL FOR [redacted]: 84 ANIMALS

[redacted]

9. [redacted]
- [redacted] 2 batches x 7 animals = 14 animals
- [redacted] 2 batches x 7 animals = 14 animals

TOTAL FOR [redacted]: 28 ANIMALS

TOTAL STUDY 1: 252

STUDY 2

[redacted]

1. [redacted] n=7 per group (Batch 13, 15); TOTAL: 2 batches x 7 animals = 14 animals
2. [redacted], n=7 per group (Batch 13, 15); TOTAL: 2 batches x 7 animals = 14 animals HOWEVER, [redacted], therefore twice as many dams are needed = 14 x 2 = 28 animals

TOTAL [redacted]: 42 ANIMALS

[redacted]

3. [redacted] from [redacted], n=3 [redacted] (Batch 15); TOTAL: [redacted] = 42 [redacted]
4. [redacted] from [redacted], n=2 [redacted] (Batch 15); TOTAL: [redacted] = 56 [redacted]

TOTAL FOR [redacted]: 98 ANIMALS

[redacted]

5. [redacted], n=7 per group (Batch 14, 16); TOTAL: [redacted] = 14 animals
6. [redacted], n=7 per group (Batch 14, 16); TOTAL: [redacted] = 14 animals [redacted] = 28 animals

TOTAL FOR [redacted]: 42 ANIMALS

[redacted]

7. [redacted] n=3 [redacted] Batch 16); TOTAL: [redacted] 42 [redacted]
8. [redacted], n=2 [redacted] (Batch 16); TOTAL: [redacted] = 56 [redacted]

TOTAL FOR [redacted]: 98 ANIMALS

[redacted]

9. [redacted] need to be counted as well:
- [redacted]: n=7 per group (Batch 14, 16); TOTAL: 2 batches x 7 animals = 14 animals
- [redacted]: n=7 per group (Batch 14, 16); TOTAL: 2 batches x 7 animals = 14 animals HOWEVER, 50% of the pups are [redacted] instead of [redacted] = 28 animals

TOTAL FOR [redacted]: 42 ANIMALS

TOTAL STUDY 2: 322

TOTAL all rats: 574 animals

References

[redacted]

Species	Origin	Maximum number of animals	Life stage
rat	[redacted]	70	[redacted]
rat		182	
rat		140	)
rat		182	

C. Re-use

Will the animals be re-used?

[] No, continue with question D.
[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Adult breeding females and males can be used for another breeding if their health and age allow it, so these animals can go back to the breeding WP. Also surplus animals may be used for the breeding of new animals for a new experiment.

Are the previous or proposed animal procedures classified as 'severe'?

[X] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
Specific (brain) mechanisms cannot be studied in vivo in humans, because of ethical restraints. Furthermore, to investigate [redacted] cannot be studied in-vitro. Therefore, it is of importance to use rats to investigate these routes.

Reduction
The requested amount of animals (based on a group size of n = 7) is needed for statistical reliable conclusions and is the minimal group size one can work with. Moreover, the same animals will be used for a variety of ex vivo studies to obtain a high number of information thereby leading to a minimal amount of animals needed.

Refinement
The experiments will be carried out with the least discomfort possible. For this reason, social housing before and during [redacted] and cage enrichment will be applied. Moreover, the analysis [redacted] propose cannot be performed without sacrificing animals. As usual, all efforts will be undertaken to minimize animal suffering during sacrifice. Only experienced researchers will handle the animals.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

[redacted] will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked twice a week to be able to detect Human End Point conditions. The animals will be weighted only during the cleaning of the cages, in this way, handling of the animals will be minimized to reduce human intervention that may cause stress for the animals. Decapitation will be performed by experienced researchers.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

N/A

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[X] No
[] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[X] No > Continue with question I.
[] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

[] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

No.

Explain why these effects may emerge.

Because the decapitation without anesthesia will be done by an experienced experimenter, and will take place in a fraction of a second, no pain or adverse effects are expected from the decapitation. As the [redacted] will be decapitated directly after the decapitation of the [redacted] their discomfort will be minimized.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

N/A

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[] No > Continue with question K.
[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

The criteria to take the animal out of the experiments are based on human observation of factors known as clear symptoms of pain/stress/discomfort and defined for humane end point detection*. Weight loss of more than 15% in one/two days and 20% over the whole study are considered as humane endpoints. Also (a combination of) general symptoms such as raised fur, hunched back (arched back), poor coat conditions, are also considered as humane endpoints after which the animals should be euthanized.
For [redacted] rats an additional humane endpoint will be applied concerning their cardiac dysfunction: a combination of clear symptoms of breathing difficulties, progressive pallor and signs of fatigue.
*Standard humane endpoints rodents: piloerection, loss of body weight (> 15%), immobility, poor self-care, tremor, self-damage, abnormal body posture, convulsions, tumors, elephant teeth.

Indicate the likely incidence.

As [redacted] is a breeding without discomfort it is unexpected that any of the animals reach the human end point over the course of the experiment (< 0%).
It is unexpected that any of the animals of [redacted] breeding with discomfort reach the human end point over the course of the experiment (< 2%).

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The total (cumulative) discomfort of the [redacted], all the dams and their [redacted] fetuses are expected to be mild. As long as these animals are not exposed to behavioral challenges they will not experience any suffering.

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

The [redacted]s of the pregnant dams are needed to analyze the brain structure and function in the brains of the [redacted] investigating changes underlying ADHD- and autism-related and co-morbid disease-related traits and to investigate changes in one or more of the three potential [redacted].
Except the [redacted]. These rats can go to the breeding protocol.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

Appendix
Description animal procedures

- This appendix should be enclosed with the project proposal for animal procedures.
- A different appendix 'description animal procedures' should be enclosed for each type of animal procedure.
- For more information, see our website www.zbo-ccd.nl.
- Or contact us by phone. (0900-2800028).

1 General information

1.1	Provide the approval number of the 'Netherlands Food and Consumer Product Safety Authority'.	10300	
1.2	Provide the name of the licenced establishment.	Stichting Katholieke Universiteit Nijmegen	
1.3	List the different types of animal procedures. Use the serial numbers provided in Section 3.4.4 of the Project Proposal form.	Serial number 3	Type of animal procedure Identification of [redacted] traits

2 Description of animal procedures

A. Experimental approach and primary outcome parameters

Describe the general design of the animal procedures in relation to the primary outcome parameters. Justify the choice of these parameters.

General design & Primary outcome measures

In the third part of this protocol [redacted] aim to determine if [redacted] show ADHD/autism-related behavioral and [redacted] traits by investigating a variety of behavioral traits in ADHD- and autism-related and co-morbid diseases-related behavioral tasks and by investigating brain function and structure through neuroimaging studies and a variety of ex vivo molecular studies in rats (Figure 8).
As it is commonly known that [redacted] can influence offspring's behavior and psychiatric liability. Thus, if [redacted] influences [redacted] this can affect our results.
Therefore, we will study the [redacted] in our studies. If the care differs due to [redacted] the pups need to [redacted]. To confound for potential differences in [redacted] levels of [redacted] of [redacted] a [redacted] need to be performed.

The offspring will be tested in one of the following groups:

[redacted] *e studies*

1. [redacted] study
2. [redacted] study

[redacted] *studies*

1. [redacted] k study
2. [redacted] study

Behavioral and cognitive measure studies

1. Autism-related traits group
2. ADHD-related traits group
3. Anxiety/depression-related traits group
4. OCD-related traits group A
5. OCD-related traits group B

Brain structure & function studies

1. Neuroimaging for brain structure and function
2. [redacted] group 1; [redacted]
3. [redacted] group 2; [redacted]
4. [redacted] group 3; [redacted]

Animals of the behavioral and cognitive measure studies and of the neuroimaging for brain structure and function group will be tested at [redacted]). Directly after the last behavior time-point/last neuroimaging procedure, rats will be sacrificed by perfusion or decapitation and their brains analyzed for changes in brain structure and function.

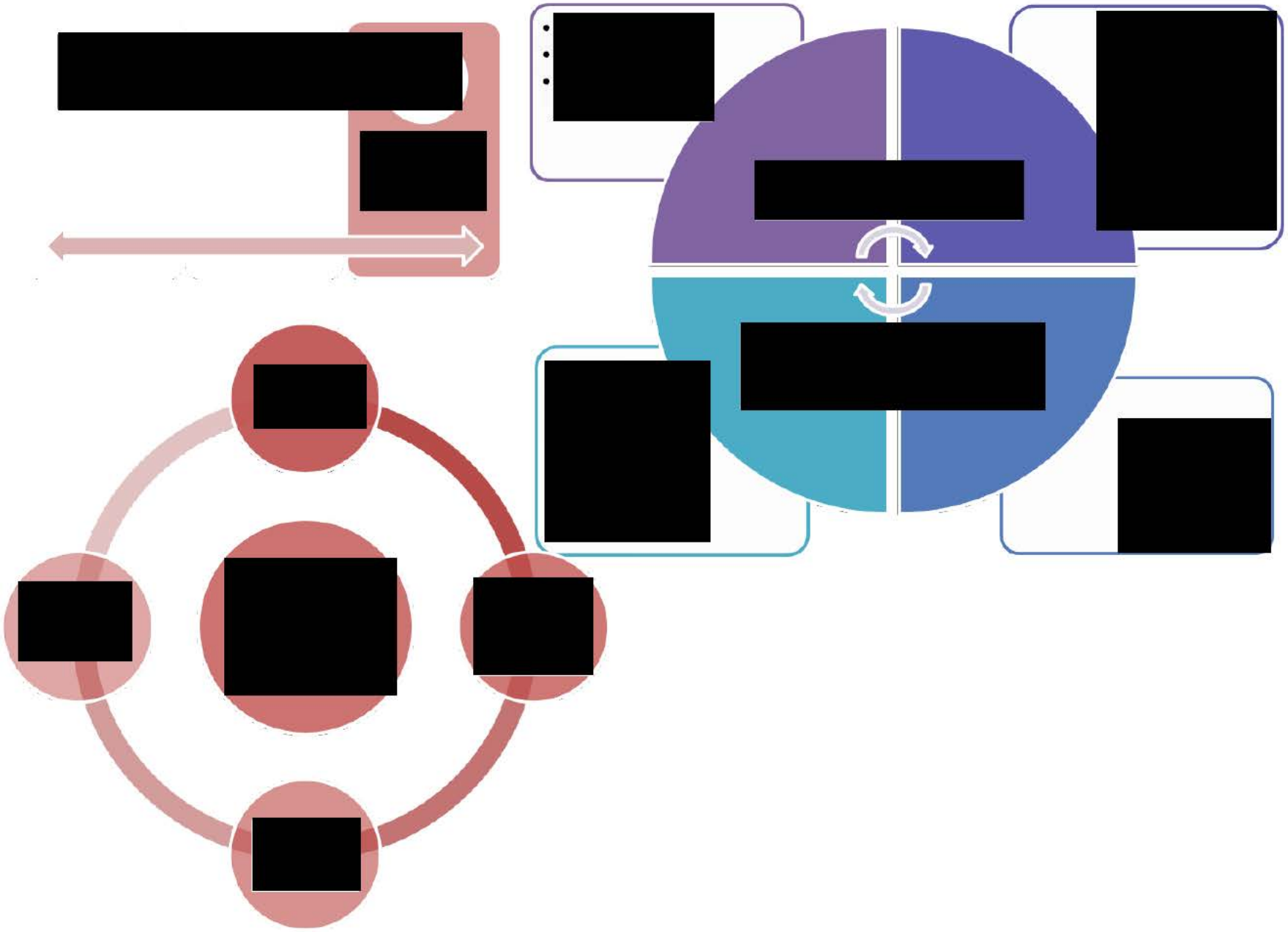


Figure 8. Research design and primary outcomes of animal procedure 1. **Left on top** experimental aim; **right on top** primary outcome measures disease-related behavioral tests **Left bottom** primary outcome measures ex-vivo tests

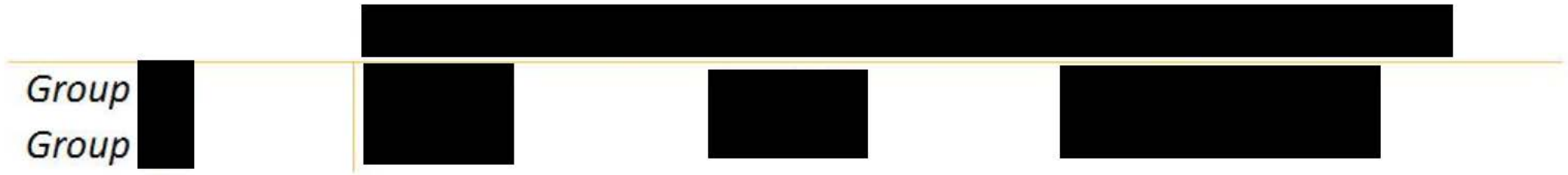
Justification:
The set of behavioral output measures is critical in order to define if [redacted] rat model shows ADHD/autism-related behavioral traits or traits related to one of the co-morbid diseases. To investigate if an animal model mimics one of the [redacted] disorder- and/or co-morbid-disorder-related traits not only behavioral clinical biomarkers (disease-related behavioral traits; DSM-5) should be compared but also biological biomarkers such as the brain structural and functional markers shown in figure 8.

Describe the proposed animal procedures, including the nature, frequency and duration of the treatment. Provide justifications for the selected approach.

[redacted] of animals
Note that in both cases, offspring all have the same [redacted] whereas [redacted] differs in the three groups. [redacted]; in case of [redacted] (described below) between [redacted] means that [redacted]) while the other [redacted] As an example, [redacted]) will be placed with a [redacted]). Therefore, when [redacted] .
[redacted] *animals*
Study 1

	[redacted]		
Group 1A	[redacted]	[redacted]	[redacted]
Group 2			

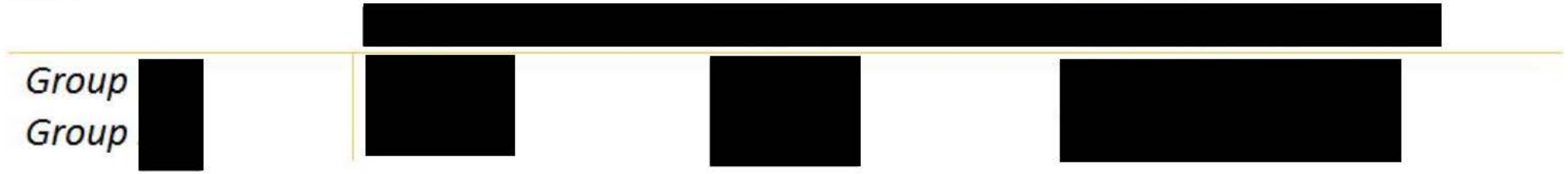
Study 2



[redacted] *animals*

Study 1

Study 2

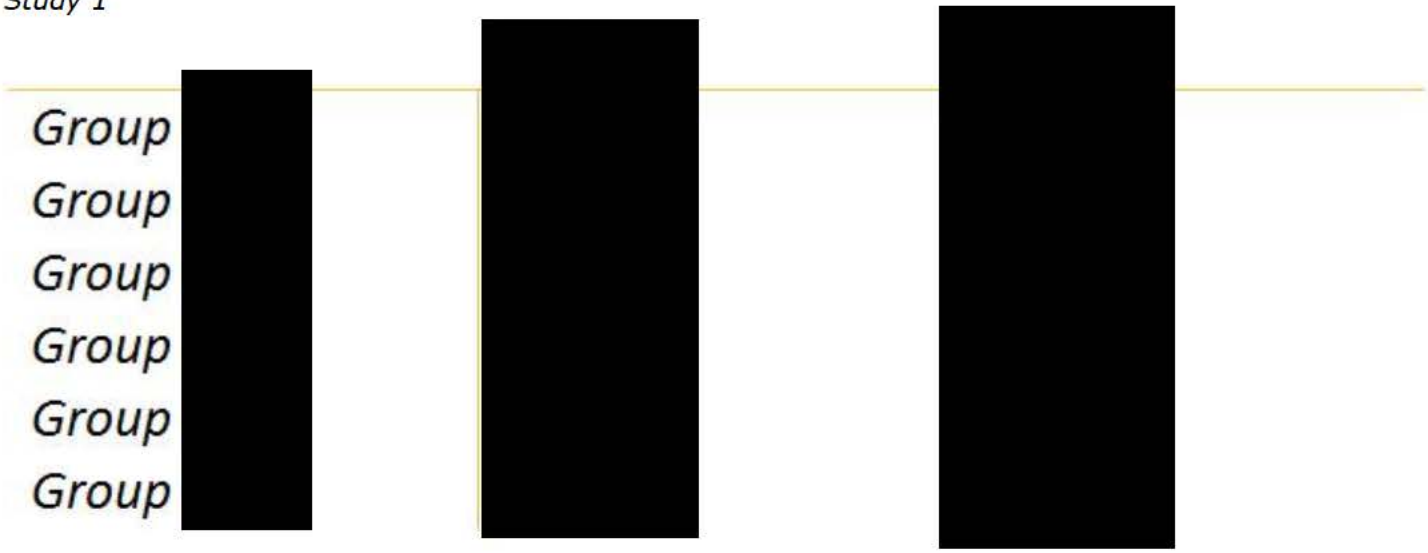


Genotyping

To determine to which group the offspring of both groups 1B belong ([redacted]) all pups need to be [redacted] Therefore ear cuts will be taken for [redacted] which can be used for the genotyping [redacted]

[redacted] will be scored in every group as a potential confounding factor. A [redacted] study needs to be performed for both [redacted] and [redacted] animal models to investigate if the [redacted] significantly differs ([redacted]). In case of [redacted] we will [redacted]. [redacted]. Thus, [redacted]
Vice versa, [redacted]
Therefore when [redacted] is needed, the amount of [redacted] and [redacted] need to be multiplied. As the levels of [redacted] in [redacted] can differ between the [redacted], the [redacted] levels in [redacted] need to be measured. To measure [redacted] levels, [redacted] has to be to be collected. Importantly, this collection is associated with a mild discomfort which may influence [redacted] intensity and thereby can indirectly impact [redacted] results. For this reason, if [redacted] will be applied, two additional groups of [redacted] will be used to study the [redacted] levels in [redacted] of [redacted] and [redacted] (additional go / no-go moment). The [redacted] of these animals will be executed at [redacted] which is [redacted]; the protocol is described below. [redacted] will be a main covariate in the statistics investigating the role of [redacted].

Groups
As the [redacted] is only an observational study only the [redacted] (and fathers) of the [redacted] will be taken into account as this is a breeding with discomfort. For the [redacted] need to be taken into account as the [redacted] need to go under anesthesia for [redacted] Group E will be used for [redacted]; group F & G will be used for the [redacted] study.
Study 1



Study 2

[redacted]

Group	[redacted]	[redacted]	[redacted]
Group			
Group			
Group			
Group			
Group			

[redacted] *scoring*

[redacted] assess [redacted] between [redacted]).

[redacted] will be scored focusing especially on three ways of [redacted]

-
-
-

[redacted])

[redacted] *collection*

If [redacted] is needed in study 1 and/or study 2, [redacted] of both [redacted] of batch F & G will be measured at the pups age of [redacted] which is [redacted] (additional go / no-go moment). A detailed protocol to [redacted]). Short summary: [redacted] A HPLC will be performed to detect [redacted]). The [redacted] will be monitored continually for signs of pain or respiratory depression and if needed the flow of isoflurane will be adjusted. The maximum time of anesthetization is 45 minutes. By using a warming chamber the [redacted] are able

to maintain proper body temperature. The [redacted] will be placed on an absorbent bench pad during recovery, after it has regained sufficient consciousness the [redacted] is placed back in the home cage with the [redacted]

Behavioral and cognitive measure studies
Male and female offspring are subjected in separate batches to the (PFC/somatosensory cortex-dependent) tests below. They are tested longitudinally across [redacted].
Due to these long lasting tests it is of importance to keep the rats active and motivated. The active phase of rats is reversed to humans; therefore, the rats will be active during the night. For this reason, the rats will be housed under a reversed dark/light cycle.

Autism-related traits group
The offspring of batch 5 ([redacted] animals) and 6 ([redacted] animals) will undergo a variety of (PFC or somatosensory cortex-dependent) behavioral tests; shown in figure 9. Using these behavioral tests [redacted] tackle six important Diagnostic and Statistical Manual of Mental Disorders (fifth edition) related criteria associated with autism: 1. social interaction (social interaction); 2. social communication (olfactory habituation/dishabituation to odors); 3. stereotyped/repetitive motor movements (novelty-induced self-grooming behavior & novel object recognition); 4. hyper- or hypo-reactivity to sensory input (prepulse inhibition and robotic gap crossing task); 5. insistence on sameness/routines (reversal learning & novel object recognition); 6. highly abnormal focus & fixated interests in unusual objects / restricted interest (object directory behavior). Lastly a control for motor development (negative geotaxis).

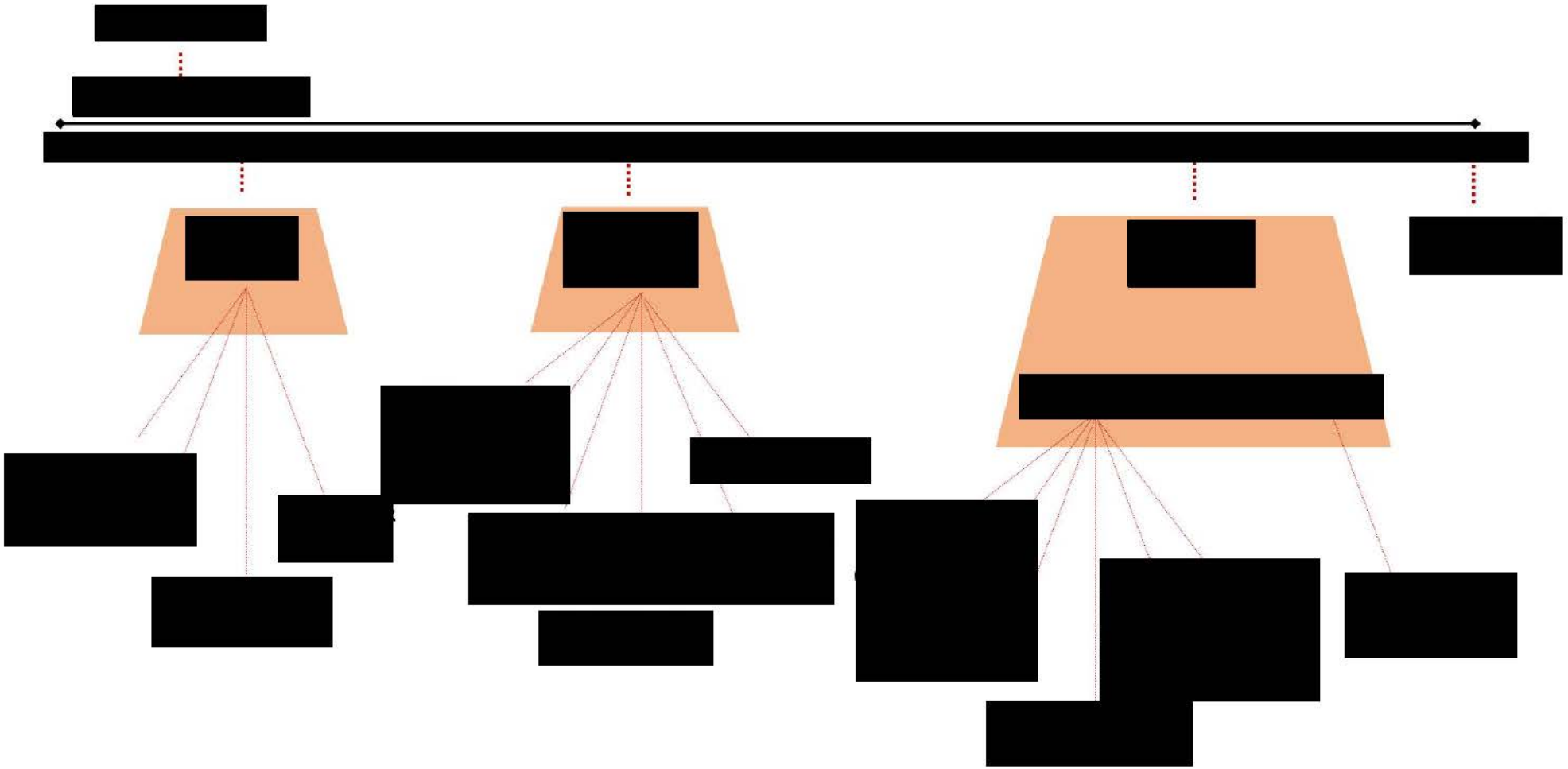


Figure 9. Timeline animal study. The [redacted] rats are exposed to a test battery at [redacted] tests in black), [redacted] (test in brown), and [redacted] (test in black and the last extensive test in brown). The test battery begins with the test left on top (an effortless test) and ends with the test right on top (the most stressful test).

Negative geotaxis
The negative geotaxis is used as a control for reflex development and is studied between [redacted]). The pup will be placed on a tilted surface (40°) with its head facing downwards and the time period until turning 180° or walking upwards is scored.

Novelty-induced grooming behavior
The grooming test studies repetitive behavior (Kalueff et al., 2016). In this test a rat will be placed into a new environment. Rats will be exposed to this paradigm at time points [redacted]. To ensure the novelty of the environment the rats will be placed in three different settings: an elevated plus maze at [redacted] an open field (circle) at [redacted] and an open field (square) at [redacted]
The rat will be placed onto the center of an elevated plus maze facing an open arm or in the center of an open field. After 300 s of freely exploring their total time (s) spent on self-grooming was scored; measuring from the start of forepaw licking until grooming ceased [redacted]). Grooming behavior is defined by Spruijt et al. (1992) as forepaw licking, face washing, scratching, body grooming and genital grooming.

Olfactory habituation/dishabituation
Olfactory discrimination measures social communication (Stack et al., 2008). Rats quickly habituate to novel odors so when an odor is presented repeatedly the rat will spend less time sniffing (habituation). If a new odor is introduced a higher level of sniffing occurs (dishabituation). In this paradigm the rat will be presented a sequence of cotton swabs containing 'social' and 'anti-social' odors; 'social' odor on the cotton swabs are obtained from urine collected from another unfamiliar rat or from the bottom of a cage of other unfamiliar rats (Stack et al., 2008) and 'anti-social' odors can be for instance almond extract or banana flavoring. The discrimination between same and different 'social' and 'anti-social' odors will be measured by scoring the time sniffing ([redacted] unpublished data). Pups will be exposed to this paradigm at time points [redacted].

Object directed behavior and Novelty object recognition
Restricted/repetitive interests (MacFabe et al., 2011) and cognition will be assessed using the object directed behavior and novelty object recognition test, respectively. Exploration of only a few objects could be analogous to restricted interests in human subjects with autism (i.e. outcome measure). Furthermore, this paradigm capitalizes on the tendency of rats to explore all aspects of a novel environment, including the sniffing of novel objects. Three objects differing in color and shape will be placed in the open field area (5 cm from the walls) and the rat will be able to explore the objects for 7 minutes. The time exploring each object is measured. After an inter-trial interval of eight hours a second trial will be performed where the object that is explored most in trial 1 is replaced by a new object. The rat will be able to explore the objects again for 7 minutes. The time exploring each object is measured. Different sets of objects will be used for the different [redacted]. Using EthoVision, the total distance moved is also measured in both tests [redacted]).

Social play and social interaction
Social play studies the interaction between rats which can be used to social interaction deficits. This test relies upon the fact that rats at their adolescence age show high social play behavior, animals showing autism-related treats will play less. During adulthood social play is diminished and replaced by normal social interactions. At both stages in life the experimental procedure is similar. Two days before the experiment the rat will already be placed for 10 minutes in the plastic cage (40×40×60 cm [l×w×h]) with approximately 2 cm of wood shavings covering the floor to habituate.
On the test day, test pairs are isolated for a maximum of 24h before the test to induce a half-maximal increase in the amount of social play behavior (Niesink & Van Ree, 1989). Two rats of similar gender, weight (difference < 10 g) and KO breed and with no social contact before will be put with each other in the cage for 15 minutes. Social play behavior and interaction of rats will be measured at P35 and P70 [redacted]).
Multiple behaviors will be scored, including:
- pinning: one of the animals lying with its dorsal surface on the floor of the test cage with the other animal standing over it
- pouncing: play soliciting by nosing the partner's nape
- boxing/wrestling: facing each other in vertical position and struggling using the forepaws

- following/chasing: moving in the direction of or pursuing the test partner, who moves away
- social grooming: licking any body part of test partner
- social exploration: sniffing any body part of the test partner

Robotic (spontaneous) Gap crossing task
Robotic gap crossing is used to study the somatosensory (whisker) function (Pang et al., 2011) of an animal which is often altered in autism. Animals are placed on one of the two elevated platforms separated from each other with randomly varying gap-distance (range: 3–8 cm, step-size: 0.5 cm) and their probability of successful object localization across gap-distances is quantified. The training is performed under infrared light and white noise. Animal mobility on the platforms is quantified using custom-made infrared motion sensors placed at the two ends and the middle of each platform. Trial duration, duration of sensory exploration at the gap, number of attempts prior to successful gap crossing, and duration of mobility are scored (Pang et al., 2011). Somatosensory (whisker) function is measured at time points [redacted].

PrePulse Inhibition
Impaired pre-pulse inhibition is a measure of sensorimotor gating (Lebow et al., 2012). To assess the animals' (latency to) peak startle and the amount of pre-pulse inhibition [redacted]. The proposed protocol is similar to those reported before (Lebow et al., 2012).
Briefly, rats are placed in a small Plexiglas cage on top of a vibration-sensitive platform in a sound-attenuated, ventilated chamber. A high-precision sensor, integrated into the measuring platform, detects movement. Two high-frequency loudspeakers inside the chamber produce all the audio stimuli. The acoustic startle response (ASR) session begins with 5 min acclimation to white background noise (70 dB) maintained through the whole session. Thirty-two startle stimuli (120 dB, 40 ms in duration with a randomly varying ITI of 12–30 s) are presented interspersed with an additional 40 startle stimuli randomly preceded by 40 ms prepulses of either 74 dB, 78 dB, or 82 dB. Maximal ASR and latency to peak startle amplitude are measured both in response to individually presented startle stimuli and in response to startle stimuli preceded by pre-pulses. Percentage pre-pulse inhibition (PPI) will be calculated as the percent difference between the maximal ASR (max G) to startle stimuli preceded by pre-pulses compared to that without.

Reversal learning
Reversal learning is used to study behavioral flexibility (Silverman et al., 2010). For this test touch screen boxes are used. Rats will establish a spatial habit, i.e. food as reinforce is given. When learned the rats need to unlearn this spatial habit to learn a new one; outcome measure is the numbers of trials needed to learn the new spatial habit.
This experiment starts at time point [redacted] The rats will be food-restricted in order to motivate performance. They will be maintained at approximately 80-90% of their free-feeding weight, starting 1 week prior to the beginning of the experiment. Rats are still socially housed. If the weight of one of the rats gets below 80% of their free-feeding weight, the animal will be additionally fed.
If there are problems with the touch screen program another paradigm will be used. Rats will establish a spatial habit, i.e. food as reinforce is given when the rat enters the left arm of a T-maze. On the test day, the day after this learning trial, rats will be placed in the T-maze again but this time the food reward is relocated to the right-arm. The numbers of visits to both the left and right arm are scored (Wöhr & Scattoni, 2013; Silverman et al., 2010).

<u>ADHD-related traits group</u>

The offspring of batch 7 (animals) and 8 (animals) will undergo a variety of (PFC or somatosensory cortex-dependent) behavioral tests; shown in figure 10. Using these behavioral tests tackle three important Diagnostic and Statistical Manual of Mental Disorders (fifth edition) related criteria associated with ADHD: 1. hyperactivity (open field test); 2. inattention (Y-maze or T-maze with four closed arms) and 3. impulsivity (five serial choice task using a touch screen).

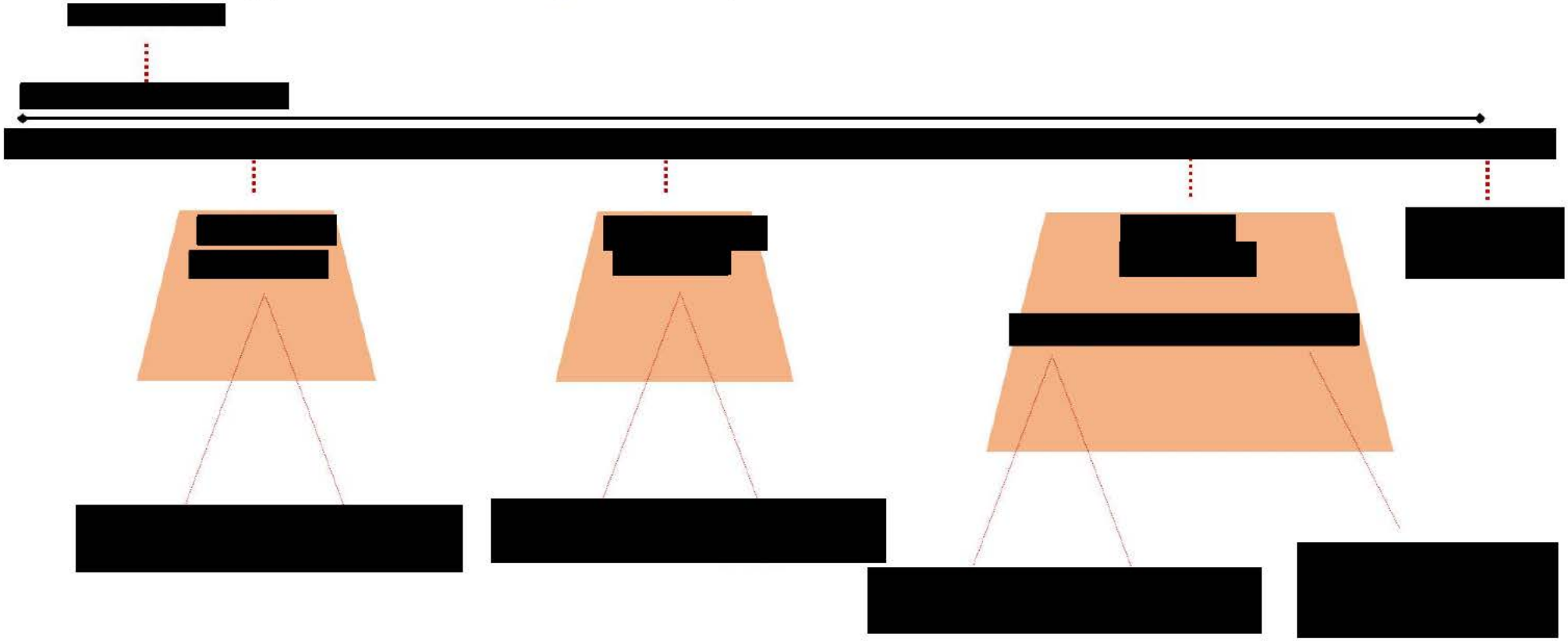


Figure 10. Timeline animal study. The rats are exposed to a test battery at (tests in black), (test in brown), and (test in black and the last extensive test in brown). The test battery begins with the test left on top (an effortless test) and ends with the test right on top (the most stressful test).

Open-field test

The open-field test is used to study hyperactivity. Rats will be placed in the corner of an open field, a squared arena (100×100×40 cm), with open top, dark walls (wood), and a dark floor (polyvinylchloride). Using a red light and a camera movement will be recorded and registered automatically for 5 minutes by EthoVision for scoring of the total distant moved ( ).

Y-maze task
The Y-maze or T-maze with four closed arms will be used to study one of the characteristics of ADHD: inattention (Botanas et al. 2016). The maze is used to study inattention as it measures the willingness to explore novel environments. Rats will be placed in one of the arms of the maze (50x10x20 cm) and can freely explore the maze for 8 min. During these 8 minutes arm entries will be monitored using EthoVision. Arm entries are defined as the entering of all four paws of the rat in the arm zone. Entering the same arm twice in a row is called a spontaneous alternation of entries. Animals showing ADHD-related traits are suspected to have a higher alternation score (%) in comparison to healthy animals.

5-CSRTT / stop signal task
Both the five-choice serial reaction time task (5-CSRTT) and stop signal task are well-known tasks measuring impulsivity (one of the characteristics of ADHD), where an animal need to perform a task to get a certain reward (e.g. food). Experiments will be conducted in rat operant chambers, if possible with a touch screen.
After habituation to the chamber and food reward (3 days max), the pre-training starts to get familiar with the touch screen; this will take a maximum of 14 days. Next the specific training starts to learn the task which will take a maximum of 30 days. Finally, the test starts which can take a maximum of 21 days; see figure 10.
Programming and data-recording are computer-controlled. The following behavioral measures will be recorded to assess task performance: (i) accuracy, i.e. percentage correct responses; (ii) latency of correct responses, i.e. the mean time between stimulus onset and a correct response; (iii) premature responses, i.e. number of responses into any of the holes during the inter-trial interval period and before stimulus onset; (iv) perseverative responses, i.e. the number of responses after correct choice during stimulus presentation or limited hold period; (v) the number of omissions, i.e. number of omitted trials during a session; and (vi) feeder latency, i.e. the latency between correct choice and collection of the food pellet ([redacted]).
This experiment starts at time point [redacted] The rats will be food-restricted in order to motivate performance. They will be maintained at approximately 80-90% of their free-feeding weight, starting 1 week prior to the beginning of the experiment. Rats are still socially housed. If the weight of one of the rats gets below 80% of their free-feeding weight, the animal will be additionally fed.

<u>Co-morbid anxiety/depression-related traits group</u>
The offspring of batch 9 [redacted] animals) and 10 ([redacted] animals) will undergo a variety of (PFC or somatosensory cortex-dependent) behavioral tests; shown in figure 11. Using these behavioral tests [redacted] tackle three important Diagnostic and Statistical Manual of Mental Disorders (fifth edition) related criteria associated with depression: 1. pessimism (ambiguous cue interpretation test), 2. psychomotor retardation (forced swim test) and 3. markedly diminished interest or pleasure in activities (sucrose preference). Moreover, the elevated plus maze is used to

study anxiety.

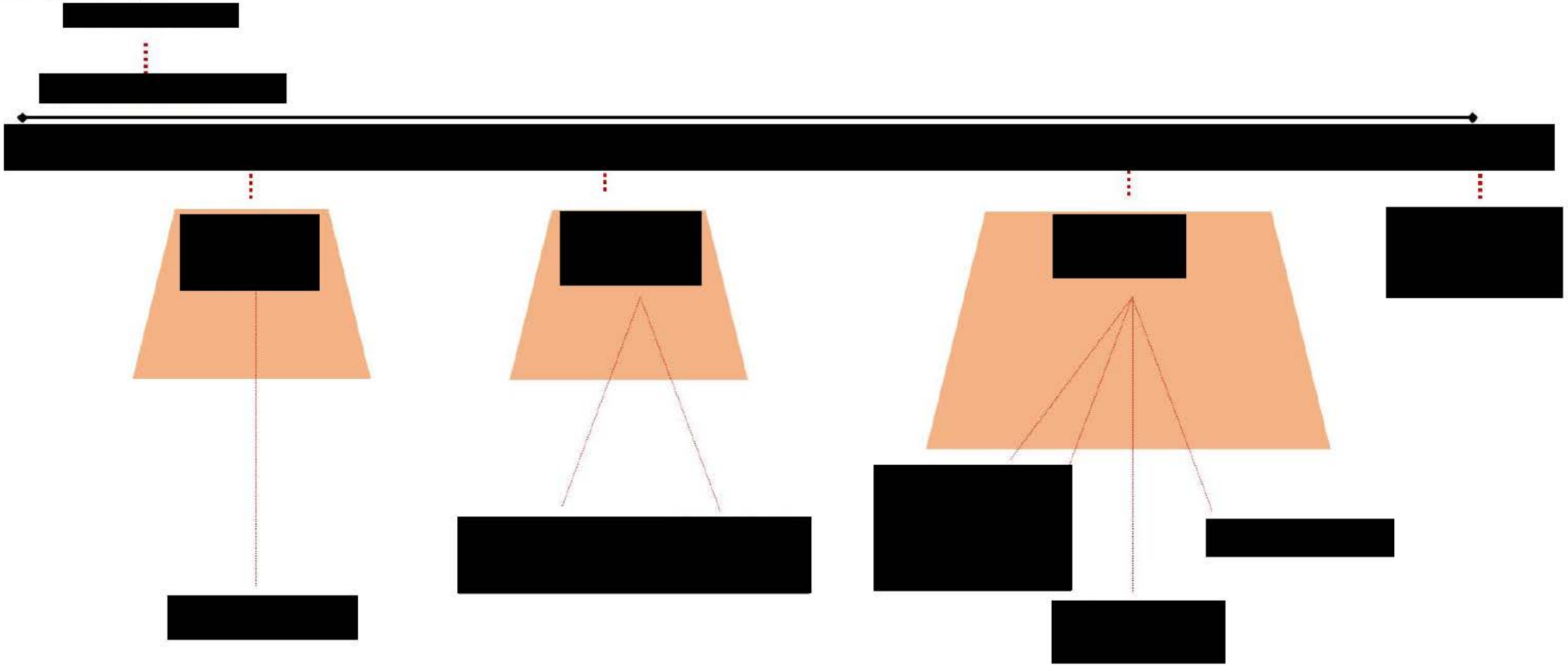


Figure 11. Timeline animal study. The [redacted] rats are exposed to a test battery at [redacted] (tests in black), [redacted] (test in brown), and [redacted] (test in black and in the middle a test in brown consisting of multiple parts). The test battery begins with the test left on top (an effortless test) and ends with the test right on top (the most stressful test).

Elevated plus maze test

The elevated plus maze is a well-characterized behavioral paradigm to define the level of anxiety-related behavior. The test relies upon the animal's natural tendency to stay in enclosed spaces and its avoidance for open spaces and heights; anxious animals will spend more time in the closed arms than less anxious animals and show a longer latency to first enter the center area or open arms. The elevated plus maze comprises a central part (5 x 5 cm), two opposing open arms (50 x 10 cm), and two opposing Plexiglas closed arms (50 x 10 x 40 cm), elevated at a height of approximately 50 cm and the open arms are illuminated with approximately 12 lux and the closed arms with approximately 4 lux. Rats are placed the center of the

maze, facing one of the open arms to initiate a 5 min test session. Time spent in the open arms, distance traveled in the open arms, and number of visits to the open arms will be quantified using a camera mounted above the apparatus and analyzed by EthoVision software (Noldus, Wageningen, Netherlands) ([redacted]). Rats need to perform this test at [redacted].

Sucrose preference
Rats receive two bottles on their cage with water or, on alternating days, one bottle is filled with water and the other one with increasing sucrose percentages solutions (2-10%). Bottles were switched on sucrose days to prevent spatial bias. Preference for the sucrose solution over water is measured; a decrease preference is indicative for anhedonia [redacted]). Rats need to perform this test at [redacted]. For this experiment, rats will be singly housed for the total of 7 days to measure the weight of both bottles.

Ambiguous cue interpretation
The ambiguous cue interpretation test is a behavioral paradigm to define pessimism (Enkel et al., 2010). This test is explained in detail by Enkel et al. (2010). In brief, in this test the animals are put in a skinner box and conditioned to two different tones (duration maximum of 9 days), one that signals a food reward when pressing on the left lever and one that signals a foot shock (±700 µA) that can be avoided by pressing the lever on the right side of the cage. After conditioning to the tones an ambiguous tone is given. The idea is that rats with positive expectations will press a lever on the left side of the cage upon hearing the ambiguous tone, whereas rats with negative expectations will choose the lever on the right side of the cage.

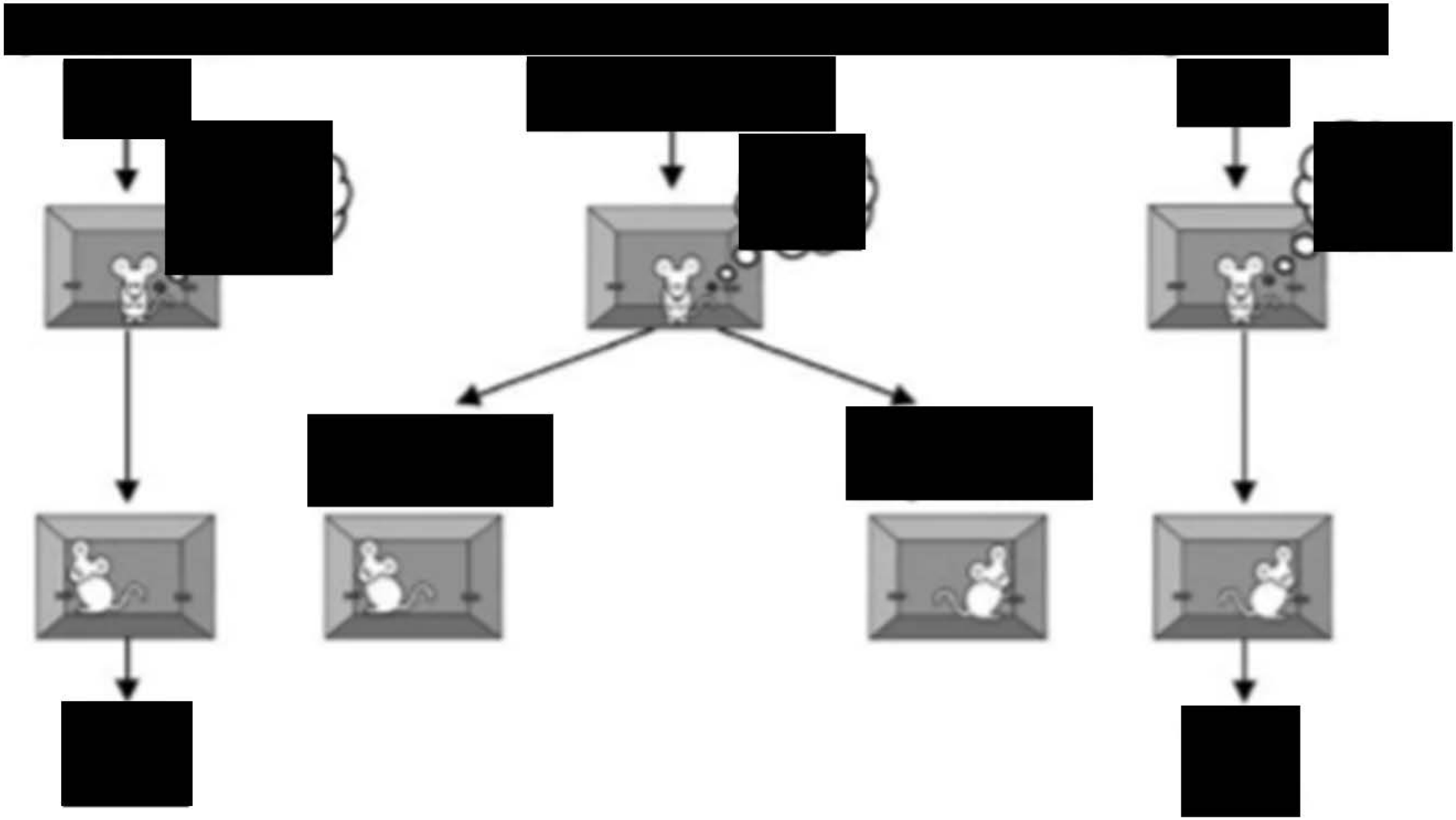


Figure 12. On the left a positive tone (2 or 9 kHz tone, counterbalanced with negative tone) signaled the opportunity to gain a reward by pressing the lever. On the right a negative tone preceded the occurance of an electric foot-shock of approximately 700 µA, which could be prevented by pressing the right lever. After discrimination training rats were tested for their responses to ambiguous tones with intermediate frequencies (3, 5 and 7 kHz). The rats'expectations of a positive or a negative event signaled by these tones were inferred from their lever responses (Enkel et al. 2010).

Forced swim test

The forced swim test is a well-characterized behavioral paradigm to define the level of psychomotor retardation. The test relies upon the animal's tendency for survival, therefore to keep swimming; depressed animals will start floating earlier and be immobile for a longer time. The forced swim test exists of two parts: the induction phase and test phase. On the induction day rats are placed in a cylinder filled with water for 15 min, on the test day rats are placed in the cylinder for 5 min [redacted]). Time spent on swimming and floating (=psychomotor retardation) is measured.

Co-morbid OCD-related traits groups

The offspring of batch 11 & 13 ([redacted] animals) and 12 & 14 ([redacted] animals) will undergo a variety of (PFC or somatosensory cortex-dependent) behavioral tests; shown in figure 13 and 14. Using these behavioral tests [redacted] tackle two important Diagnostic and Statistical Manual of Mental Disorders (fifth edition) related criteria associated with OCD: 1. sensory compulsivity (schedule-induced polydipsia procedure) and motor compulsivity (signal attenuation task).

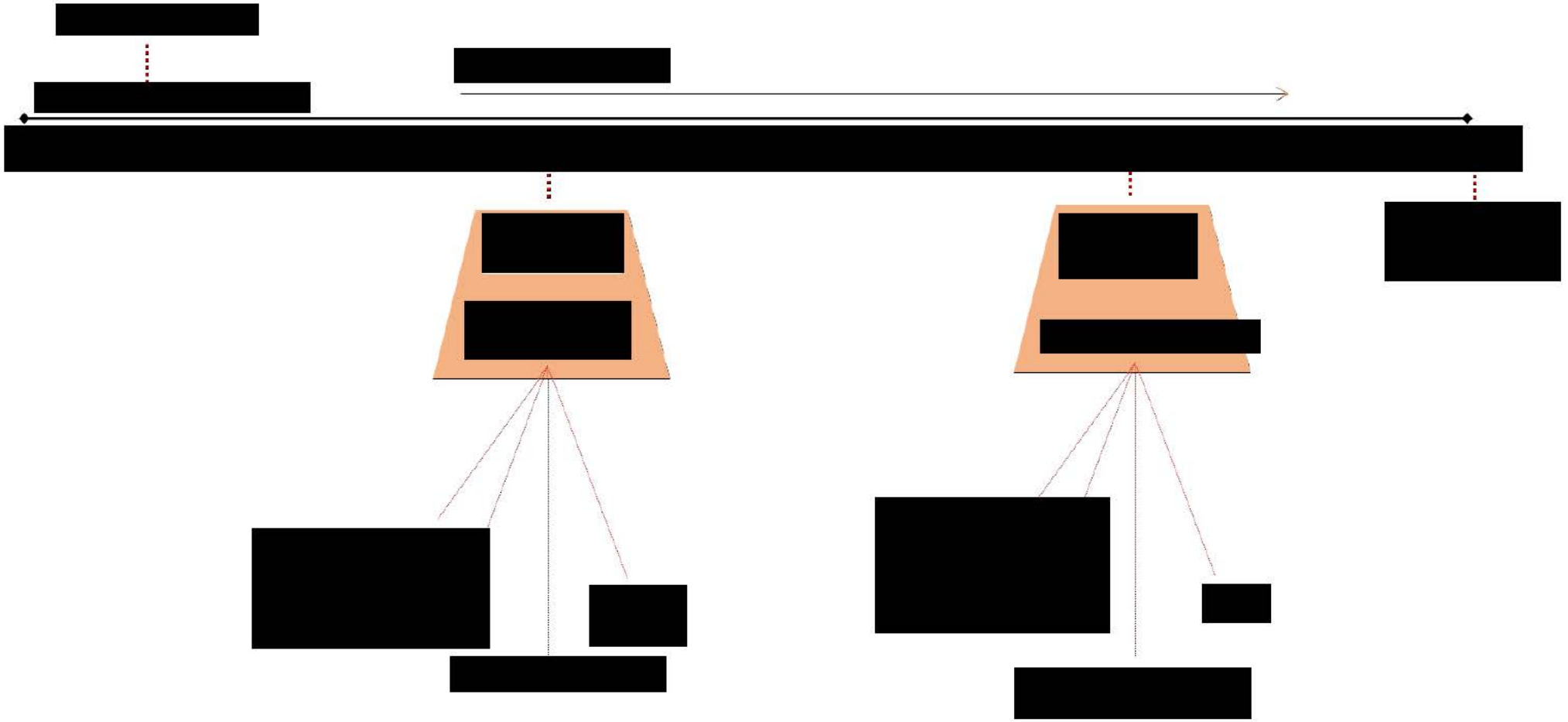


Figure 13. Timeline animal study. The [redacted] rats are exposed to a test at [redacted] (test in black), and [redacted] (test in brown).

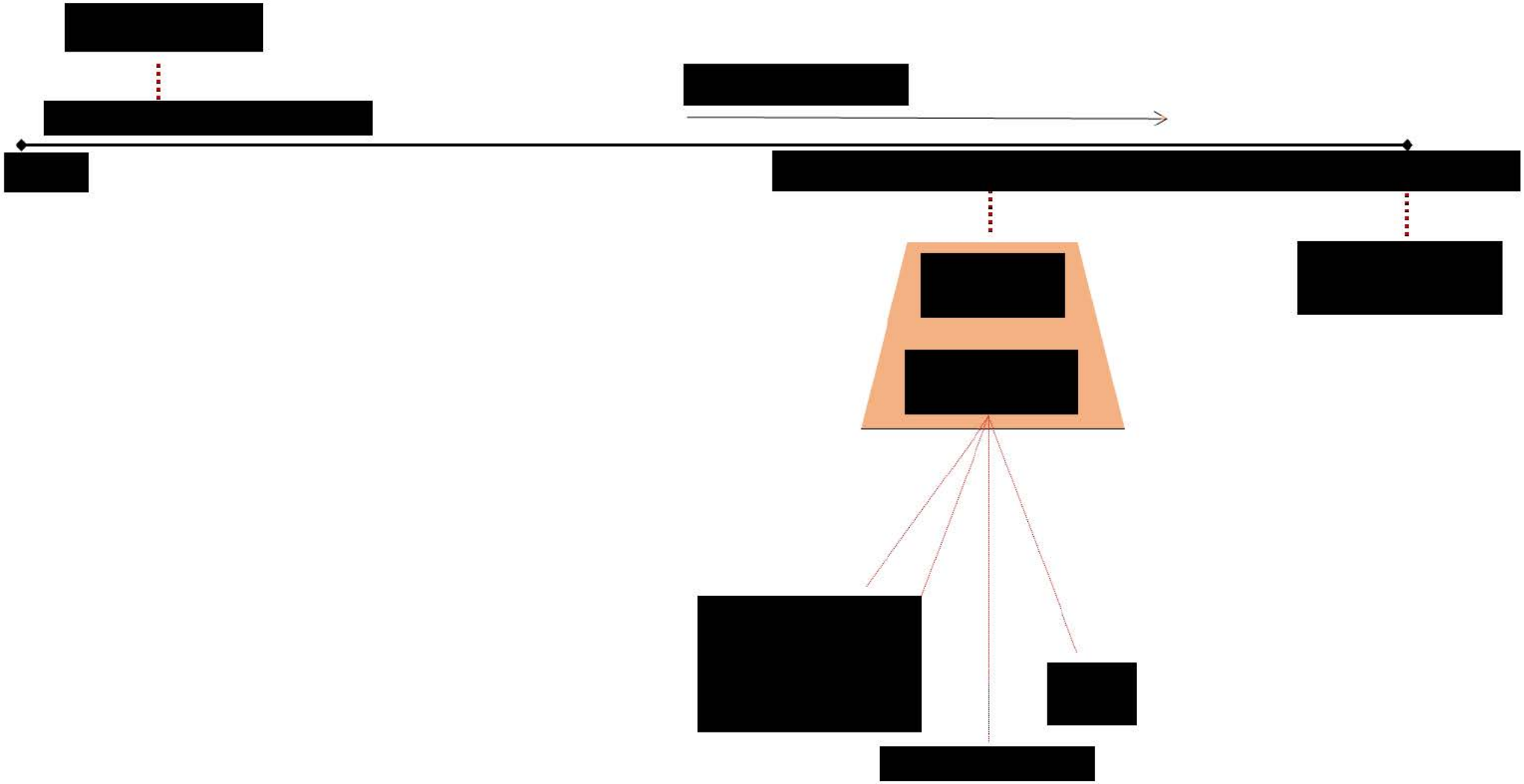


Figure 14. Timeline animal study. The [redacted] rats are exposed to a test at adult behavior.

Schedule-induced polydipsia procedure
The schedule-induced polydipsia procedure is a paradigm to study sensory compulsivity but also impulsivity (Ibias & Pellón, 2011). It is a maladaptive, excessive intake of freely available water resulting in a decrease in corticosterone levels (Dantzer et al 1988) in the face of predictable intermittent food delivery that has been suggested to generate distress in animals (Platt et al., 2008).

For this procedure rats will be placed in operant chambers and exposed to a series of fixed time (FT) schedules. Different schedules can be used meaning that using different time intervals (e.g. 30, 60, 180 s) food pellets will be delivered regardless of the animal's behavior. As water will be available at all times the amount of water drunk will be increased, thereby inducing polydipsia (Ibias & Pellón, 2011).
This experiment starts at time point [redacted]. The rats will be food-restricted in order to motivate performance. They will be maintained at approximately 80-90% of their free-feeding weight, starting 1 week prior to the beginning of the experiment. For this experiment, rats will be singly housed.

Signal attenuation task
The signal attenuation task is a paradigm to study motor compulsivity. The experiment will be conducted in rat operant chambers. Programming and data-recording are computer-controlled. This paradigm is explained in detail by Schilman et al. (2010). In brief, this paradigm exists of 4 steps, first a training will be given to collect the food pellet; second a lever-press training will be performed where pressing on one of the two levers will lead to the drop of a food pellet; third the signal attenuation training will be performed to unlearn the food reward – stimulus connection (learned in the first training) and fourth the test where the rats can press a lever but only the stimulus and not the food reward will be presented. The following behavioral measures will be scored: the number of lever presses on the RL after the first response (extra lever presses, ELP) in uncompleted trials (that is, ELP not followed by magazine entry; ELP-U) and ELP in completed trials (that is, ELP followed by magazine entry, ELP-C) (Schilman et al., 2010). This experiment will be performed once at [redacted]
This experiment starts at time point [redacted] The rats will be food-restricted in order to motivate performance. They will be maintained at approximately 80-90% of their free-feeding weight, starting 1 week prior to the beginning of the experiment. Rats are still socially housed. If the weight of one of the rats gets below 80% of their free-feeding weight, the animal will be additionally fed.

<u>Sacrifice</u>
After the experiment male and female offspring are perfused with 4% paraformaldehyde or decapitated (figure 6 – 8 and 10, 11) to investigate brain function and structure through a variety of measurements. The ex-vivo measurements are described in the next session 'Brain structure & function studies'

Brain structure & function studies
<u>Neuroimaging for brain structure and function</u>
The [redacted] of batch 15 ([redacted] animals) and 16 ([redacted] animals) will be imaged at the age of [redacted]; undergoing e.g. sMRI, fMRI, ASL and DTI.
All animals will undergo three MRI sessions. The rats will be placed in a stereotactic device with ear bars and tooth holder to immobilize the head. Body temperature will be measured using a rectal thermometer and maintained at 37 °C using a heated air flow device. The respiration rate was registered using a breathing pad. To prevent dehydration, a special eye ointment was used for the eyes.

The rats will be anesthetized with 3.5% isoflurane and transferred to the MRI platform, where anesthesia levels are reduced to 2%. The rats subsequently receive a bolus of medetomidine (Dexdomitor, 0.05 mg/kg) subcutaneously (Grandjean et al., 2014). After five minutes, the isoflurane will be further reduced to 1%, and another five minutes later it will be lowered to 0.5%, and infusion of medetomidine (0.1 mg/kg/h) started (Grandjean et al., 2014) which will be maintained throughout the scanning session to maintain the superficial sedation level.

The animals will undergo ~2.5 hrs of MRI scanning. Two arterial spin labeling (ASL) scans are acquired at 45 to 60 minutes after the medetomidine bolus. After completion of scanning, the animals will be removed from the apparatus, halting the administration of isoflurane and the medetomidine infusion, and a bolus (0.25 mg/kg) of antisedan (Atipamezole) will be administered subcutaneously to antagonize the medetomidine and ensure quick recovery (Adamczak et al., 2014).

After the experiment male and female offspring are perfused with 4% paraformaldehyde or decapitated to investigate brain function and structure through a variety of measurements.

Naïve groups

As explained earlier, three separated naïve groups will be studied as well to investigate brain structure & function at infancy, adolescence and adulthood without influences due to the behavioral tasks (i.e. stress and food restriction).

- [redacted] group for [redacted]; batch 17 ([redacted] animals) and 18 ([redacted] animals)
- [redacted] group for [redacted]; batch 19 ([redacted] animals) and 20 ([redacted] animals)
- [redacted] group for [redacted]; batch 21 [redacted] animals) and 22 [redacted] animals)

Ex vivo: Prefrontal and somatosensory cortical architecture

Balance between inhibitory and excitatory signals in PFC

Coronal sections are double stained for NeuN (all neurons) and GAD67 (glutamic acid decarboxylase 67, marks GABA-ergic neurons). NeuN+ and GABA+ neurons are inhibitory, NeuN+ and GABA- neurons non inhibitory, probably excitatory.

Hippocampal neurogenesis activity

A variety of staining's are used, for proliferation Ki67 and BrdU; for differentiation DCX; stemness Sox2 and Nestin; cell death CC3; neurons Map2 and astrocytes GFAP.

Formation of the [redacted] *network*

Coronal sections are stained for [redacted] (special [redacted]); [redacted] as a marker for [redacted] in [redacted]

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Describe which statistical methods have been used and which other considerations have been taken into account to minimise the number of animals.

Throughout all procedures data dropout and loss of animals will be minimized by careful execution of the experiments and close monitoring animal welfare. As a rat is exposed to multiple behavioral studies and used for ex-vivo tests. [redacted] estimate that [redacted] need maximally 15 rats per group per gender; based on previous work ([redacted]). We will calculate the precise group sizes per experiment using a power analysis (power 90% and alfa of 0.05), based on data collected so far by us and others.

References

[redacted]

Go / no-go moment & analyses: between [redacted] & analyses study 1 & 2

As multiple behavior data is scored it is of importance to make a division between primary and secondary analyses. The primary analyses investigate if the most important criteria's of a [redacted] disorder or co-morbid disorder is significantly different between the groups. The secondary analyses investigates other DSM criteria's of a disorder to get a more detailed idea of the influence of the [redacted] on the [redacted].

The [redacted] plays a role in the [redacted] when only primary criteria's of ADHD and autism are significantly different in comparison to the control group. When also the primary criteria's of both co-morbid disorders (anxiety/depression and OCD) are significantly changed the [redacted] seems to play a more broader way concerning psychological disorders.

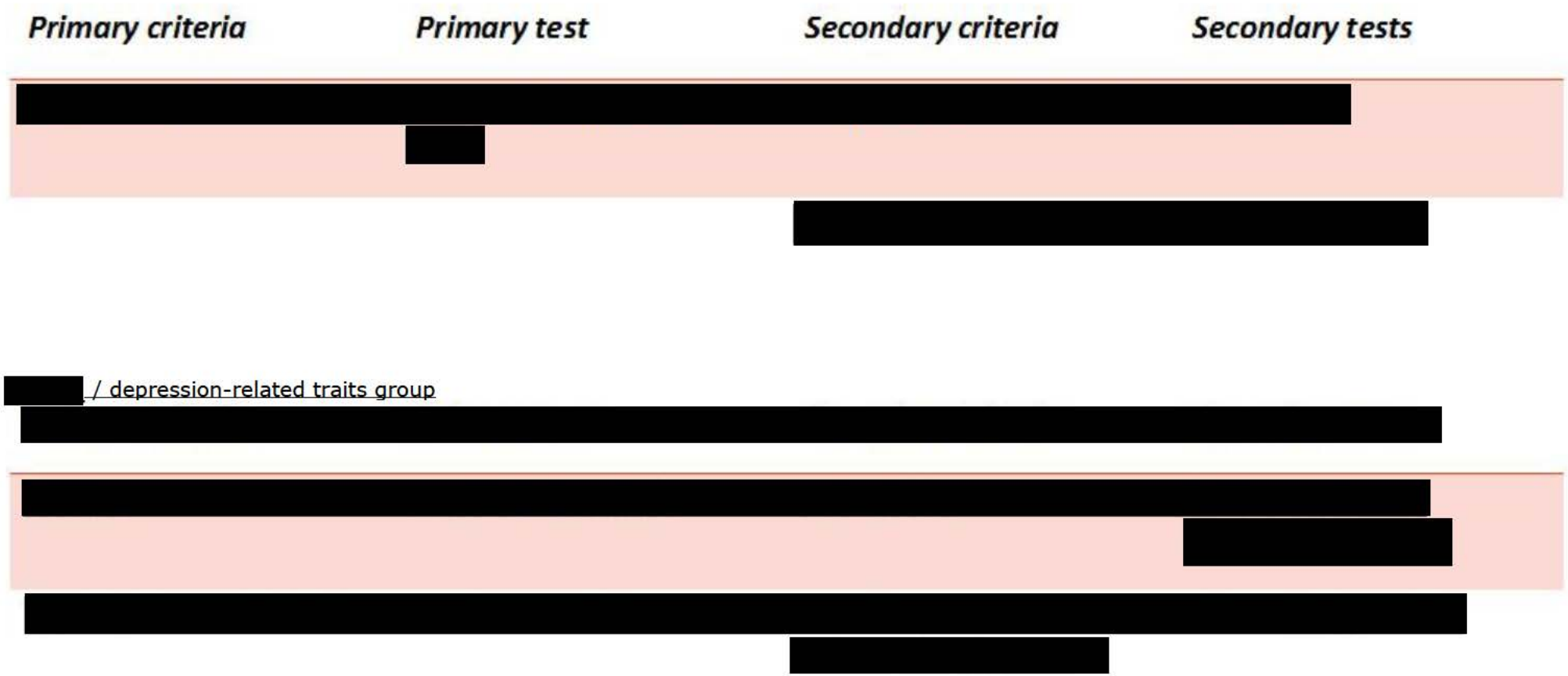
When the primary criteria's of only one disorder, [redacted] or co-morbid, is significantly different the [redacted] seems to be play a more specific role concerning that disease.

Only when all primary criteria's of a disease are significantly different between the groups the behavioral test group will be studied again in study 2. Only brain structural and functional changes, studied ex vivo and neuroimaging, which were found significant different between the two groups will be investigated in study 2. Furthermore, only the gender in which this significant effect was found will be studied further.

Behavioral and cognitive measure studies

[redacted]-related traits group

[redacted]-related traits group


Primary criteria
Primary test
Secondary criteria
Secondary tests
[redacted] / depression-related traits group
[redacted]-related traits group

Primary criteria	Primary test	Secondary criteria	Secondary tests
[redacted]			

B. The animals

Specify the species, origin, estimated numbers, and life stages. Provide justifications for these choices.

[redacted] use the [redacted]) and [redacted]) [redacted] rats to model the [redacted] respectively. [redacted] use these [redacted] rats as [redacted] models for [redacted] disorders investigating ADHD and autism traits (see background information). [redacted] rats exhibit high [redacted] levels in body and brain, and [redacted] rats [redacted] [redacted] in the body. The [redacted] genetic rat line is bred by [redacted]. The [redacted] rats are provided by [redacted], further breeding towards [redacted] will be also [redacted].
As gender differences play a role in the pathogenesis of these disorders, male and female offspring will be used.This difference is not only shown in human studies but also by an earlier study where female [redacted] rats showed increased anxiety in comparison to males [redacted]

References

[redacted]

The breeding of [redacted] animals is a breed without any discomfort. The breeding of [redacted] animals is however a breed with discomfort as the null mutants from [redacted] crosses develop cardiac insufficiency in adulthood [redacted]).

Table 3. Animal batches per study per animal model.

Study	*Sub studies*	[redacted] *model*	[redacted] *animal model*
[redacted]	-	-	Batch 0
[redacted] ***studies***	-	-	Batch E
[redacted] ***studies***	-	Batch F	Batch G
[redacted] ***studies***	[redacted] group	Batch 5	Batch 6
	[redacted] group	Batch 7	Batch 8
	[redacted] group	Batch 9	Batch 10
	[redacted] group 1	Batch 11	Batch 12
	[redacted] group 2	Batch 13	Batch 14
[redacted] ***studies***	[redacted]	Batch 15	Batch 16
	Naive group; [redacted]	Batch 17	Batch 18
	Naive group; [redacted]	Batch 19	Batch 20
	Naive group; [redacted]	Batch 21	Batch 22

STUDY 1
The following groups are proposed for each gender:
[redacted] *studies (Batch E)*
1. [redacted], n=10 per group (Batch E)
2. [redacted], n=10 per group (Batch E)
TOTAL FOR [redacted] STUDIES: 20 ANIMALS
[redacted] *studies (Batch F & G)*

1. [redacted] n=5 per group (Batch F)
2. [redacted], n=5 per group (Batch F)

1. [redacted], n=5 per group (Batch G)
2. [redacted], n=5 per group (Batch G)
TOTAL FOR [redacted] STUDIES: 20 ANIMALS

[redacted] *studies (Batch 1 to 10 so 5 batches per animal model)*
5. [redacted] animals from [redacted], n=15 per gender per group (Batch 1, 3, 5, 7, 9); TOTAL: 2 gender x 5 batches x 15 animals = 150 animals;
however when [redacted] n=30 per gender (n=15 per gender for [redacted] [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 5 batches x 30 animals = 300 animals
6. [redacted] animals from [redacted], n=15 per gender per group (Batch 1, 3, 5, 7, 9); TOTAL: 2 gender x 5 batches x 15 animals = 150 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 5 batches x 30 animals = 300 animals
7. [redacted] animals from [redacted], n=15 per gender per group (Batch 2, 4, 6, 8, 10); TOTAL: 2 gender x 2 studies x 5 batches x 15 animals =150 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 5 batches x 30 animals = 300 animals
8. [redacted] animals from [redacted], n=15 per gender per group (Batch 2, 4, 6, 8, 10); TOTAL: 2 gender x 5 batches x 15 animals = 150 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 5 batches x 30 animals = 300 animals
TOTAL FOR [redacted] EXPERIMENTS: 1200 ANIMALS (without [redacted] 600); meaning 240 animals per behavioral group (autism, ADHD, anxiety, OCD A and OCD B).

[redacted] *studies (Batch 11 to 18)*

9. [redacted] animals from [redacted], n=15 per gender per group (Batch 11, 13, 15, 17); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;
however when [redacted] n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 4 batches x 30 animals = 240 animals
10. [redacted] animals from [redacted], n=15 per gender per group (Batch 11, 13, 15, 17); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 4 batches x 30 animals = 240 animals
11. [redacted] animals from [redacted], n=15 per gender per group (Batch 12, 14, 16, 18); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 4 batches x 30 animals 240 animals
12. [redacted] animals from [redacted] mothers, n=15 per gender per group (Batch 12, 14, 16, 18); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 4 batches x 30 animals = 240 animals
TOTAL FOR [redacted] EXPEIRMENTS: 960 ANIMALS (without [redacted]: 480); meaning 240 animals per group (neuroimaging, [redacted]).

[redacted] *(Batch 0)*
1. [redacted] group 1a:
1 female [redacted] x 1 male [redacted] = 6 [redacted] [redacted] (males & females)
è A total of 15 [redacted] males is needed for the [redacted].
è A total of 360 [redacted] pups is needed for [redacted] experiments (batch 6, 8, 10, 12, 14) and 180 [redacted] pups for [redacted] experiments (batch 16, 18, 20, 22). 540 pups divided by 6 pups per breed = 90 females [redacted] and 90 males [redacted] needed.
Total of 195 animals
2. [redacted] group 2:
1 female [redacted] x 1 male [redacted] = 6 [redacted] [redacted] offspring (males & females)
è A total of 15 [redacted] males is needed for the [redacted] and [redacted] studies.
è A total of 360 [redacted] pups is needed for [redacted] (batch 6, 8, 10, 12, 14) and 180 [redacted] pups for [redacted] experiments (batch 16, 18, 20, 22). 540 pups needed divided by 6 pups per breed = 90 females [redacted] and 90 males [redacted] needed.
Total of 195 animals
TOTAL FOR [redacted]: 390 ANIMALS

TOTAL STUDY 1: 2.590

STUDY 2
The following groups are proposed for each gender:
[redacted] *studies (Batch E)*

1. [redacted], n=10 per group (Batch E)
2. [redacted], n=10 per group (Batch E)
TOTAL FOR [redacted] STUDIES: 20 ANIMALS
[redacted] *studies (Batch F & G)*

1. [redacted], n=5 per group (Batch F)
2. [redacted], n=5 per group (Batch F)

1. [redacted], n=5 per group (Batch G)
2. [redacted], n=5 per group (Batch G)
TOTAL FOR [redacted]: 20 ANIMALS

[redacted] *studies (Batch 1 to 10 so 5 batches per animal model)*
19. [redacted] animals from [redacted] mothers, n=15 per gender per group (Batch 1, 3, 5, 7, 9); TOTAL: 2 gender x 6 batches x 15 animals = 180 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted] mother); TOTAL: 2 gender x 6 batches x 30 animals = 360 animals
20. [redacted] animals from [redacted], n=15 per gender per group (Batch 1, 3, 5, 7, 9); TOTAL: 2 gender x 6 batches x 15 animals = 180 animals;
however when [redacted]g: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 6 batches x 30 animals = 360 animals
21. [redacted] animals from [redacted], n=15 per gender per group (Batch 2, 4, 6, 8, 10); TOTAL: 2 gender x 5 batches x 15 animals = 150 animals;
however when [redacted] n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 5 batches x 30 animals = 300 animals
22. [redacted] animals from [redacted], n=15 per gender per group (Batch 2, 4, 6, 8, 10); TOTAL: 2 gender x 5 batches x 15 animals = 150 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted] mother); TOTAL: 2 gender x 5 batches x 30 animals = 300 animals
TOTAL FOR [redacted] EXPERIMENTS: 1200 ANIMALS (without [redacted] 600); meaning 240 animals per behavioral group (autism, ADHD, anxiety, OCD A and OCD B).

Brain structure & function studies (Batch 11 to 18)
23. [redacted] animals from [redacted], n=15 per gender per group (Batch 11, 13, 15, 17); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted] TOTAL: 2 gender x 4 batches x 30 animals = 240 animals
24. [redacted] animals from [redacted], n=15 per gender per group (Batch 11, 13, 15, 17); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;

however when [redacted] n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 4 batches x 30 animals = <u>240 animals</u>
25. [redacted] from [redacted], n=15 per gender per group (Batch 12, 14, 16, 18); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of [redacted] and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 4 batches x 30 animals = <u>240 animals</u>
26. [redacted] from [redacted] mothers, n=15 per gender per group (Batch 14, 16, 18); TOTAL: 2 gender x 4 batches x 15 animals = 120 animals;
however when [redacted]: n=30 per gender (n=15 per gender for [redacted] of own mother and n=15 per gender for [redacted] of [redacted]); TOTAL: 2 gender x 4 batches x 30 animals = <u>240 animals</u>
TOTAL FOR [redacted]: 960 ANIMALS (without [redacted]: 480); meaning 240 animals per group (neuroimaging, [redacted]).

[redacted] *(Batch 0)*
1. [redacted] 1b:
1 female [redacted] x 1 male [redacted] = 3 [redacted] (males & females) and 3 [redacted] which are not involved in the experiment
è A total of 15 [redacted] is needed for the [redacted] studies.
è A total of 360 [redacted] is needed for [redacted] (Batch 6, 8, 10, 12, 14) and 180 [redacted] pups for [redacted] experiments (Batch 16, 18, 20, 22). Important: 50% of the [redacted] instead of [redacted], therefore twice as many [redacted] are needed then normal. Total: 540 pups needed divided by 3 pups per [redacted] = 180 females [redacted] and 180 males [redacted] needed.
<u>Total of 375 animals</u>
2. [redacted] 2:
1 female [redacted] x 1 male [redacted] = 6 [redacted] (males & females)
è A total of 15 [redacted] males is needed for the [redacted] and [redacted] studies.
è A total of 360 [redacted] is needed for [redacted] experiments (Batch 6, 8, 10, 12, 14) and 180 [redacted] for [redacted] experiments (Batch 16, 18, 20, 22). Total: 540 pups needed divided by 6 pups per breed = 90 females [redacted] and 90 males [redacted] needed.
<u>Total of 195 animals</u>
TOTAL FOR [redacted]: 570 ANIMALS
TOTAL STUDY 2: 2.770

<u>TOTAL all rats: 5.360 animals</u>

Species	Origin	Maximum number of animals	[redacted]
rat	[redacted]	2	
rat		2	
rat		1	
rat		20	

C. Re-use

Will the animals be re-used?

[] No, continue with question D.
[X] Yes > Explain why re-use is considered acceptable for this animal procedure.

Adult [redacted] can be used for another breeding if their health and age allow it so these animals can go back to the breeding WP. Also surplus animals may be used for the breeding of new animals for a new experiment. The females for the [redacted] and [redacted] studies can be re-used in the breeding protocol.

Are the previous or proposed animal procedures classified as 'severe'?

[X] No
[] Yes > Provide specific justifications for the re-use of these animals during the procedures.

D. Replacement, reduction, refinement

Describe how the principles of replacement, reduction and refinement were included in the research strategy, e.g. the selection of the animals, the design of the procedures and the number of animals.

Replacement
The rat is the best animal model to perform behavioral studies to investigate [redacted]. Because of the complexity of these kind of disorders, it is impossible to use lower-order animals. Furthermore, since it is a [redacted] disorder [redacted] can study the onset and development of disease traits from an early age on. Specific brain mechanisms and behaviors cannot be studied in vivo in humans, because of ethical restraints. Also, the life line of humans is very long, which hampers longitudinal studies in humans.

Reduction
The requested amount of animals (based on a group size of n = 15) is needed for statistical reliable conclusions and is the minimal group size one can work with. Furthermore, the same animals will be used for a variety of behavioral paradigms to obtain a high number of information thereby leading to a minimal amount of animals needed.

Refinement
The experiments will be carried out with the least discomfort possible. For this reason, social housing and cage enrichment will be applied as long as

possible. When housed alone, rats will be placed in the same room so they can still see/hear/smell each other. Only experienced researchers will perform the experiments.

Explain what measures will be taken to minimise 1) animal suffering, pain or fear and 2) adverse effects on the environment.

The discomfort [redacted] rats will be exposed to is limited to the absolute minimum necessary to answer [redacted] research questions. Animals will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked on a daily basis to be able to detect Human End Point conditions. At a young age the animals will be weighted daily which will decline to once a week from adolescent age onwards. Noteworthy, food restricted rats maintained at approximately 80-90% of their free-feeding weight will be weighted daily as well. Moreover, rats undergoing neuroimaging measurements will be maintained and monitored continuously using MRI-compatible instruments. Vital parameters include temperature, O2 saturation and heart rate. Given the non-invasive nature of MRI studies, health is not expected to be affected.
Handling of the animals will be minimized to reduce human intervention that may cause stress for the animals. Perfusion will take place under deep anesthesia to minimize adverse effects.

Repetition and Duplication

E. Repetition

Explain what measures have been taken to ensure that the proposed procedures have not already been performed. If applicable, explain why repetition is required.

Not applicable

Accommodation and care

F. Accommodation and care

Is the housing and care of the animals used in experimental procedures not in accordance with Annex III of the Directive 2010/63/EU?

[] No
[X] Yes > If this may adversely affect animal welfare, describe how the animals will be housed and provide specific justifications for these choices.

The rats exposed to schedule-induced polydipsia procedure (batch 7 – 10) will be singly housed during this task. The rats exposed to the sucrose preference task will be single housed during this task (Batch 9 & 10).

G. Location where the animals procedures are performed

Will the animal procedures be carried out in an establishment that is not licenced by the NVWA?

[X] No > Continue with question H.
[] Yes > Describe this establishment.

Provide justifications for the choice of this establishment. Explain how adequate housing, care and treatment of the animals will be ensured.

Classification of discomfort/humane endpoints

H. Pain and pain relief

Will the animals experience pain during or after the procedures?

[] No > Continue with question I.
[X] Yes > Will anaesthesia, analgesiaor other pain relieving methods be used?

[] No > Justify why pain relieving methods will not be used.

[X] Yes > Indicate what relieving methods will be used and specify what measures will be taken to ensure that optimal procedures are used.

The discomfort [redacted] rats will be exposed to is limited to the absolute minimum necessary to answer [redacted] research questions. Animals will be monitored daily and closely by the caretakers and scored individually for signs of discomfort and checked on a daily basis to be able to detect Human End Point conditions and weighted once a week. Handling of the animals will be minimized to reduce human intervention that may cause stress for the animals. Rats undergoing neuroimaging tasks will be anesthetized for ±3 hours. In the beginning the rats will be anesthetized with 3.5% isoflurane, subsequently they receive a bolus of medetomidine. After five minutes, the isoflurane will be reduced to 1%, and another five minutes later it will be lowered to 0.5%. Infusion of medetomidine (0.1 mg/kg/h) started which is maintained throughout the scanning session. [redacted] undergoing milk

collection will be anesthetized using 5% isoflurane (flow 1.000 cc per min) which will be lowered to 2-3% isoflurane when anesthetization is confirmed.

I. Other aspects compromising the welfare of the animals

Describe which other adverse effects on the animals welfare may be expected?

Some of the behavioral tests (e.g. ambiguous cue interpretation test, elevated plus maze) are (also) stressful at the psychological level. Some of the behavioral tests (e.g. Forced Swim Test, ambiguous cue interpretation test) are stressful, but mostly at the psychological level, less at the level of pain. Only the ambiguous cue interpretation test can result in physically pain due to the use of foot shocks. The idea is however that the amount of foot shocks will decrease over time as the rats will learn to avoid it by pressing a lever. Furthermore, due to stress after surgery, rats may be more afraid to human contact. However, after 3 days of handling, these rats show normal behavior again. The animal's welfare may also be affected by the lack of a shelter or cage mates.

Explain why these effects may emerge.

In the forced swim test the rats are placed in a cylinder with water without escape possibilities. This causes substantial psychological stress. Rats keep their head above the water surface by either swimming or floating. In the ambiguous cue interpretation test the rats can lever press to avoid shocks. They initially may fail and receive the shocks. When they have acquired the tasks shock exposure will be low. These stressors are however necessary for these experiments to succeed. Another example is the slight food deprivation which animals will undergo when tested on the signal attenuation task, schedule-induced polydipsia procedure or signal stop task/5CSRTT. This animals will be food deprived as a sucrose pellet is used as motivator/reward or to induce polydipsia. In addition, in a variety of behavioral tests (e.g. Novelty-induced grooming behavior; open-field test) the rats will be placed in novel environments which can increase anxiety. These stressors are however necessary for these experiments to succeed. Moreover, rats need to be anesthetized before undergoing a neuroimaging task to diminish the stress for the animals during the scanning; furthermore it is necessary for successful images that the rats lay still.

Indicate which measures will be adopted to prevent occurrence or minimise severity.

Although some stressors are inherent to the experimental design, [redacted] will take precautionary measures to minimize all other potential causes of (additional) stress to the animals, e.g., by socially housing the rats with cage enrichment and only partial cleaning of the housing cages to retain hierarchy (and thereby prevent fighting to re-establish this hierarchy).

J. Humane endpoints

May circumstances arise during the animal procedures which would require the implementation of humane endpoints to prevent further distress?

[] No > Continue with question K.

[X] Yes > Describe the criteria that will be used to identify the humane endpoints.

The criteria to take the animal out of the experiments are based on human observation of factors known as clear symptoms of pain/stress/discomfort and defined for humane end point detection*. Weight loss of more than 15% in one/two days and 20% over the whole study are considered as humane endpoints. Also (a combination of) general symptoms such as raised fur, hunched back (arched back), poor coat conditions, are also considered as humane endpoints after which the animals should be euthanized. [redacted] will contact a veterinarian if there is doubt.
For [redacted] rats an additional humane endpoint will be applied concerning their [redacted]: a combination of clear symptoms of breathing difficulties, progressive pallor and signs of fatigue.
*Standard humane endpoints rodents: pilo-erection, loss of body weight (> 15%), immobility, poor self-care, tremor, self-damage, abnormal body posture, convulsions, tumors, elephant teeth.
** If the animal is under food restriction resulting in 80-90% weight loss, the human endpoint of weight loss is defined as 15 or 20% weight loss in comparison to their food restriction weight.

Indicate the likely incidence.

It is unexpected that any of the animals reach the human end point over the course of the experiment. (< 2%).

K. Classification of severity of procedures

Provide information on the expected levels of discomfort and indicate to which category the procedures are assigned (non-recovery, mild, moderate, severe).

The total (cumulative) discomfort of 3.440 rats (64.2%) is expected to be mild.
The total (cumulative) discomfort of 1.920 rats (35.8%) is expected to be moderate due to the behavioral paradigm or due to the anesthesia for neuroimaging.

Table 4. Experimental set-up possibilities and total discomfort outcomes.

Animals	[redacted]				
[redacted] study n = 40 (0.8%) batch E	study	-	-	-	mild
[redacted] n = 40 (0.8%) batch F & G			-	-	mild
[redacted] groups n = 480 (9.0%) batch 5 & 6	5 tests	7 tests **1 hour:** Single housing **Prepulse Inhibition:** Startle	8 tests **1 hour:** Single housing **Prepulse Inhibition:** Startle **P85 – P123:** food restriction	Decapitation / Perfusion	moderate
[redacted] groups n = 480 (9.0%) batch 7 & 8	2 tests	2 tests	3 tests **P75 – p150:** food restriction	Decapitation / Perfusion	mild
[redacted] groups n = 480 (9.0%) batch 9 & 10	1 test	1 test **Sucrose test:** single housing	3 test **Sucrose test:** single housing **Ambiguous Cue test:** Shock	Decapitation / Perfusion	moderate
[redacted] A groups n = 480 (9.0%) batch 11 & 12	-	1 test **P35 – p47:** food restriction + single housing	1 test **P70 – p90:** food restriction + single housing	Decapitation / Perfusion	moderate
[redacted] B groups	-	-	1 test	Decapitation / Perfusion	mild

End of experiment

L. Method of killing

Will the animals be killed during or after the procedures?

[] No > Continue with Section 3: 'Signatures'.
[X] Yes > Explain why it is necessary to kill the animals during or after the procedures.

The brains of the animals are needed to analyze the brain structure and function investigating changes underlying ADHD- and autism-related and co-morbid disease-related traits.
Except the [redacted] and the mothers for the [redacted] and [redacted]. These rats can go to the breeding protocol.

Is the proposed method of killing listed in Annex IV of Directive 2010/63/EU?

[] No > Descripe the method of killing that will be used and provide justifications for this choice.

[X] Yes

DEC-advies

A. Algemene gegevens over de procedure

1. Aanvraagnummer: 2016-0039
2. Titel van het project: [redacted] matters! [redacted] and [redacted] effects
3. Titel van de NTS: Nieuwe inzichten in de rol van serotonine in psychische ontwikkelingsstoornissen; een belangrijke rol voor serotonine van de moeder

4. Type aanvraag:
   ☒ nieuwe aanvraag projectvergunning
   ☐ wijziging van vergunning met nummer

5. Contactgegevens DEC:
   - naam DEC: RUDEC
   - telefoonnummer contactpersoon: [redacted], bereikbaar op maandag, dinsdag, en donderdag van 9:00 tot 15:00 uur
   - e-mailadres contactpersoon: [redacted]!
   -

6. Adviestraject (data dd-mm-jjjj):
   ☒ ontvangen door DEC: 15-09-2016
   ☐ aanvraag compleet
   ☒ in vergadering besproken: 04-10-2016, 08-11-2016 en 06-12-2016
   ☐ anderszins behandeld
   ☒ termijnonderbreking(en) van 11-10-2016 tot 18-10-2016 en van 14-11-2016 tot 22-11-2016
   ☐ besluit van CCD tot verlenging van de totale adviestermijn met maximaal 15 werkdagen
   ☒ aanpassing aanvraag: 17-10-2016 en 23-11-2016
   ☒ advies aan CCD: 29-12-2016

7. De inhoud van dit project is afgestemd met de IvD en deze heeft geen bezwaren tegen de uitvoering van het project binnen deze instelling.

8. Eventueel horen van aanvrager:
   - Datum
   - Plaats
   - Aantal aanwezige DEC-leden
   - Aanwezige (namens) aanvrager
   - Gestelde vraag / vragen
   - Verstrekt(e) antwoord(en)
   - Het horen van de aanvrager heeft wel/niet geleid tot aanpassing van de aanvraag.

9. Correspondentie met de aanvrager
   - Datum: 11-10-2016 en 18-10-2016
   - Gestelde vragen en antwoorden:
     Project Proposal:
     -3.1 In de subparagraaf 'The [redacted] hypothesis, [redacted] wordt in de eerste zin de [redacted] hypothese genoemd. De commissie meent dat de aanvrager de [redacted] hypothese bedoelt.
     Antwoord: Veranderd in de [redacted] project aanvraag.

-3.1 Er worden drie manieren beschreven waarlangs [redacted] (of [redacted] daarvan) invloed uitoefenen op de hersenontwikkeling van nageslacht in de baarmoeder. Is dit van belang voor het bewijzen van de [redacted]
Antwoord: Het uitzoeken welke mechanismen onderliggend zijn aan de neurotrofe effecten van het [redacted] is van belang om de hypothese te bewijzen. Door onderliggende mechanismen aan te tonen wordt er op een andere manier bewezen dat deze hypothese aanneembaar is; er wordt zo gezegd een extra draagvlak gecreëerd. Daarnaast maakt het weten van deze mechanismen het mogelijk om specifieke targets voor te stellen als nieuwe biomarkers en als nieuwe 'druggable targets'.
-3.2: De informatie over de te volgen strategie om de doelstelling te behalen hoort thuis bij vraag 3.4.1 ('to answer ….& functional traits'). De haalbaarheid van de doelstelling is nog niet onderbouwd met verwijzingen naar eigen publicaties.
Antwoord: De informatie over de te volgen strategie is verplaatst naar 3.4.1. Verder is er een publicatie toegevoegd om de haalbaarheid van de doelstelling te onderbouwen.
-3.4.1 De experimenten die de onderzoekers willen doen om onderzoeksvraag 3 [redacted] te onderzoeken zijn nog niet uitgebreid genoeg beschreven. De commissie kan uit de huidige tekst niet opmaken hoe de resultaten van de voorgestelde experimenten zullen bewijzen welk van de [redacted] relevant is in vivo. Kunnen de onderzoekers dit in samenhang met figuur 2 verduidelijken?
Antwoord: [redacted] hebben de tekst uitgebreid met een tabel waarin uitgelegd staat per [redacted] en [redacted] welke veranderingen er in het [redacted] en [redacted] van de experimentele groep te meten moeten zijn ten opzichte van de controle groep.
-3.4.1 De onderzoekers zullen de experimenten met [redacted] dieren achterwege laten indien zij geen bewijs vinden voor de [redacted] hypothese . Kunnen de onderzoekers de criteria duidelijker omschrijven voor dit go / no go moment? Welk resultaat is afdoende bewijs voor de geldigheid van de hypothese?
Antwoord: De nakomelingen van de twee groepen worden op veel verschillende manieren met elkaar vergeleken. Om deze reden heb ik bij de statistieken van de DAP (punt 3) een overzicht gecreëerd waarin is aangegeven welke DSM criteria en de daarbij behorende testen het belangrijkste zijn om aan te duiden of het diermodel deze ziekte representeert (primaire criteria & test) en welke criteria en testen als secundair worden beschouwd.
Alleen wanneer aan alle primaire criteria van een ziektebeeld wordt voldaan zal de tweede studie van start gaan met betrekking tot die ziekte.
-3.4.2/3.4.3: De aanvrager zal het gedrag van de ontwikkelende pups onderzoeken met betrekking tot symptomen die gerelateerd zijn aan vier ziektebeelden. Hebben alle testen hetzelfde gewicht, of zijn sommige testen belangrijker voor het meten van bijvoorbeeld autisme of ADHD? Kunnen de onderzoekers duidelijker omschrijven wanneer zij een significant hoofdeffect bewezen achten? Welke testresultaten zijn cruciaal voor dit bewijs? (zie ook de vraag over 3.4.1)
Antwoord: Zoals hierboven beschreven worden de nakomelingen van de twee groepen inderdaad op veel verschillende manieren met elkaar vergeleken. Om deze reden heb ik bij de statistieken van de DAP (punt 3) een overzicht gecreëerd waarin is aangegeven welke DSM criteria en de daarbij behorende testen het belangrijkste zijn om aan te duiden of het diermodel deze ziekte representeert (primaire criteria & test) en welke criteria en testen als secundair worden beschouwd. De secundaire testen zijn van belang om een gedetailleerd beeld te creëren van de rol van het [redacted] op de ziekte.
Alleen wanneer aan alle primaire criteria van een ziektebeeld wordt volstaan kan er worden aangenomen dat het [redacted] een rol speelt bij de ontwikkeling van die ziekte.
Er zijn verschillende uitkomsten mogelijk:

- Wanneer er alleen op 1 ziekten significante effecten worden gevonden op de primaire criteria dan kan worden aangenomen dat het [redacted] effecten een rol speelt bij een specifieke ziekte.
- Wanneer er alleen significante effecten worden gevonden op de primaire criteria van ADHD en autisme dan kan worden geconcludeerd dat het [redacted] via [redacted] effecten alleen een rol speelt bij de ontwikkeling van [redacted]
- Wanneer er significante effecten worden gevonden op alle primaire criteria van alle onderzochte ziekten, [redacted] en hun co morbide ziekten, kan er worden aangenomen dat het [redacted] een rol speelt bij de ontwikkeling van meerdere psychische stoornissen.
-3.4.3 Het betreft een omvangrijke projectaanvraag vanwege een zeer relevante vraagstelling. De uitwerking hiervan in de DAPs is niet goed navolgbaar voor de DEC, ondermeer door de veelheid aan testen die nodig is. Kunnen de onderzoekers een gelimiteerde aanpak voorstellen waarin het proof of concept wordt aangetoond alvorens aan dit grote project te beginnen? Het effect van [redacted] i**n deze ratmodellen** op [redacted] en gedrag van pups is nog niet eerder gepubliceerd. De commissie is daarom van mening dat een extra go / no go moment in deze projectaanvraag op zijn plaats zou zijn . De commissie verzoekt u alvorens te starten met dit omvangrijke project eerst een kleiner experiment te doen waarmee dit effect wordt aangetoond bij pups waarin dit effect het duidelijkst zal zijn. Zij verwacht dat experimenten met mannelijke pups van [redacted] hiervoor volstaan, maar laat het design hiervan graag aan de experts op dit terrein. Wanneer dit effect (onderzoeksvragen 1 & 2) is aangetoond in gedragsexperimenten, dan kan het mechanisme onderzocht worden en later ook studie 2.
Antwoord: Allereerst wil ik u erg bedanken voor uw meedenken. Ik ben het met u eens dat dit een omvangrijke projectaanvraag is met veel testen en hoop dat door het inbrengen van extra structuur de DAPs beter te volgen zijn (vraag A2, B en K).
Ik begrijp dat eerst het 'proof of concept' moet zijn aangetoond voordat er aan zo een omvangrijke studie kan worden begonnen. Naar onze mening is deze 'proof of concept' echter al via eerdere humane en dieren studies aangetoond; studies toegevoegd in PP 3.1 en beschreven hieronder. Meerdere humane studies laten een link zien tussen het [redacted] veranderingen in het brein en de ontwikkeling van [redacted] zoals ADHD en autisme. Daarnaast hebben recente muizenstudies aangegeven dat [redacted] heeft op het [redacted] van de foetus en daardoor de [redacted] beïnvloed. Deze informatie is de basis van de [redacted] welke wij in onze aanvraag verder willen onderzoeken door de onderliggende mechanismen te bestuderen en door een link te leggen naar ontwikkelingsziekten en hun co morbide ziekten.
Beschreven in PP 3.1:
"We recently conducted a human pilot study and reported that [redacted] affected the [redacted] of children. We compared MRI scans and cognitive performance of N=35 children of [redacted] to those of N=44 children of [redacted]. All children themselves carried the [redacted]. Somatosensory cortex grey matter density and fine motor task performance were 1.5- to 2.5-times greater in children of [redacted] compared to children of LL mothers, thereby showing that the [redacted] can affect child's phenotype (Van der Knaap et al., 2014). In another human pilot study family analysis of 38 [redacted] and 41 of their offspring revealed that offspring of [redacted] exhibited 1.5- to 2.5-timeshigher ADHD scores and related symptoms than did controls or offspring of fathers with the corresponding [redacted] (Halmøy et al., 2010). Moreover, a recent article of [redacted] suggests a link between [redacted] and the risk for autism.

*Focusing on animal data, mouse fetuses that were fully capable of producing [redacted] but conceived by [redacted], had an abnormally shaped cortex ([redacted] More importantly, recent studies from Bonnin his research group ([redacted] showed in mice that the [redacted] has an effect on [redacted]. **Thus** [redacted] **has profound effects on** [redacted] **in** [redacted] **However, what the underlying** [redacted]-[redacted] **processes are is currently unknown**. Noteworthy, [redacted]) did not find evidence linking [redacted] of the children themselves to ADHD."*

Description of Animal Procedures:

*DAP1

-A1: Testen voor OCD en Angststoornissen worden toegevoegd, maar ontbreken in de gegeven achtergrond van het projectvoorstel.

Antwoord: OCD en angststoornissen zijn beide toegevoegd aan de gegeven achtergrond van het projectvoorstel.

-A1: De verschillen tussen de twee groepen nakomelingen worden met elkaar vergeleken door middel van een groot aantal testen. Op welke manier zullen de onderzoekers deze resultaten interpreteren? Welke conclusie wordt bijvoorbeeld getrokken indien slechts één of twee van de zeven testen voor ADHD-symptomen positief blijken?

Antwoord: Zoals hierboven beschreven worden de nakomelingen van de twee groepen inderdaad op veel verschillende manieren met elkaar vergeleken. Om deze reden heb ik bij de statistieken van de DAP (punt 3) een overzicht gecreëerd waarin is aangegeven welke DSM criteria en de daarbij behorende testen het belangrijkste zijn om aan te duiden of het diermodel deze ziekte representeert (primaire criteria & test) en welke criteria en testen als secundair worden beschouwd. De secundaire testen zijn van belang om een gedetailleerd beeld te creëren van de rol van het [redacted] op de ziekte.

-A2: Er wordt hier en in de tabel bij K verwezen naar batches 1-18. Kunnen de onderzoekers een duidelijker overzicht geven van alle batches?

Antwoord: In DAP: deel B is er een overzichtstabel toegevoegd met de uitleg waar alle batches naar verwijzen.

-A2: In de beschrijving van de marble burying test is sprake van muizen.

Antwoord: De term 'muizen' was inderdaad onjuist. Gezien deze test voornamelijk bij muizen wordt uitgevoerd hebben [redacted] uiteindelijk toch besloten 'marble burying' uit de testbatterij te halen.

-B: Alle experimenten worden in mannen en vrouwen gedaan. Is het mogelijk om met mannelijke dieren te beginnen, aangezien ADHD en autisme vaker bij mannen voorkomen, en sleutelexperimenten eventueel te herhalen in het andere geslacht?

Antwoord: ADHD en autisme hebben inderdaad een hogere prevalentie bij mannen maar om dit verschil extra te kunnen benadrukken is het ook van belang om vrouwtjes te gebruiken. Bovendien zal het uitstellen van het testen van vrouwelijke pups leiden tot een verhoogd aantal surplus dieren per test, eerst zullen alle vrouwelijke pups als surplus dieren worden beschouwd en later zullen alle mannelijke pups als surplus dieren worden beschouwd. Daarnaast zal er twee keer zoveel moeten worden gefokt wat zal zorgen voor een hoger aantal [redacted] dieren met mogelijk ongerief.

Om deze redenen verzoeken wij om de experimenten in vrouwen en mannen tegelijkertijd te kunnen starten.

-B: De beschrijving van de groepen en aantallen waarbij studies 1 en 2 zijn samengevoegd is nauwelijks te volgen. Kunnen de aanvragers dit overzicht beter leesbaar aanleveren?

Antwoord: Door een extra tabel met de uitleg van alle batches toe te voegen en de tekst onder te verdelen in de verschillende studies: [redacted] studies', 'behavioral and

cognitive measure studies, 'brain structural & functional studies', 'breeding', is dit overzicht beter leesbaar geworden. Daarnaast zijn deze termen ook gebruikt in de PP en in de DAP: A2 en K.

-I: Stress na de operatie wordt genoemd als bron van ongerief. Welke operatie bedoelt de aanvrager hier?

Antwoord: Mijn excuses dit had nog uit de tekst weggehaald moeten worden gezien [redacted] geen operatie gepland hebben; dit is nu gewijzigd in het nieuwe projectvoorstel.

-I: De fok van de [redacted] dieren is een fok met ongerief vanwege hartfalen op volwassen leeftijd. Zijn alle benodigde fokdieren meegeteld in de projectaanvraag? Vanaf welke leeftijd ontstaat dit ongerief en hoe wordt dit ongerief vermeden wanneer een dier wordt hergebruikt voor de fok? (Geldt ook voor DAP2)

Antwoord: Alle benodigde fokdieren zijn nu meegeteld in de projectaanvraag.

Gezien dit de eerste keer zal zijn dat [redacted] ratten worden gebruikt is er momenteel alleen nog muizen data gericht op het ongerief van deze dieren. In de artikelen van [redacted] ([redacted]) wordt aangegeven dat deze muizen op volwassen leeftijd een verhoogde kans hebben op hartfalen en daarbij problemen kunnen krijgen met de ademhaling, vermoeid zijn en progressieve bleekheid kunnen vertonen.

In deze studie en fok zal er voor de zekerheid rekening worden gehouden met de mogelijkheid dat deze problemen ook bij ratten voor kunnen komen. Wanneer een rat symptomen vertoond (moeilijke ademhaling, weinig beweging en progressieve bleekheid) zal het dier uit de studie worden gehaald.

-J: Eén van de humane eindpunten is 20% gewichtsverlies, terwijl dit nodig is voor het uitvoeren van een gedragsexperiment (voedselrestrictie tot 80-90% van hun normale gewicht).

Antwoord: [redacted] hebben deze informatie toegevoegd: op het moment dat een dier onder voedselrestrictie is zal de 15 en 20% gewichtsverlies berekend worden met hun voedselrestrictie gewicht als baseline.

-J: Wat is het humane eindpunt voor de [redacted] dieren (in de fok en misschien ook in het experiment)? (zie tweede vraag over I) (Geldt ook voor DAP2, hier staat ten onrechte dat het een fok zonder ongerief is)

Antwoord: In deze studie en fok zal er voor de zekerheid rekening worden gehouden met de mogelijkheid dat deze problemen ook bij ratten voor kunnen komen. Wanneer een rat symptomen vertoond (moeilijke ademhaling, weinig beweging en progressieve bleekheid) zal het dier uit de studie worden gehaald. Dit is toegevoegd aan beide DAPs.

Mijn excuus, in DAP 2 (nu omgezet naar DAP 1) staat nu ook dat het om een fok met ongerief gaat.

-K: Is de ongeriefinschatting van de polydipsie-test adequaat? Kortstondig solitair opsluiten in kooien van volwassen ratten is inderdaad licht ongerief, maar is 12 dagen solitaire huisvesting van adolescente ratten en 20 dagen van volwassen ratten op voedselrestrictie tot 80-90% van hun normale gewicht nog licht ongerief? De test is opgezet om een effect op corticosteron-spiegels te meten.

Antwoord: Ik ben het met u eens dat alle handelingen afzonderlijk voor licht ongerief zullen zorgen maar door de combinatie van 12 dagen solitaire huisvesting en 32 dagen voedselrestrictie 12 dagen adolescente leeftijd + 20 volwassen leeftijd) het totale ongerief voor deze dieren matig zal zijn. Om deze reden is het ongerief veranderd in het nieuwe projectvoorstel van licht naar matig.

-K: De berekening van het percentage dieren met mild of matig ongerief is niet goed navolgbaar met de gegeven tabel (zie ook vraag A2 en B).

Antwoord: Reactie: In B is een extra tabel toegevoegd welke uitleg geeft over de betekenis van de batches. De termen van deze tabel komen nu overeen met de uitleg in A2 en B.

*DAP2

-A2: De moeders worden onder anesthesie geperfundeerd, waarbij de pups ook geperfundeerd worden. Zijn de pups ook onder anesthesie door de anesthesie van de moeder, en zullen de onderzoekers dit checken?
Antwoord: Dit is inderdaad een belangrijke vraag. In de literatuur [redacted] *en* [redacted] *) wordt gebruikt gemaakt van deze techniek maar hierbij wordt niet vermeld of de pups ook geperfundeerd worden en hoe dit wordt gecheckt. Om deze reden hebben* [redacted] *besloten de methode te wijzigen. De moeders en de pups zullen gedecapiteerd worden, dit zal zo snel mogelijk achter elkaar plaatsvinden. Direct na de decapitaties zullen de breintjes van de pups geperfundeerd worden door ze overnacht in 4% paraformaldehyde te leggen.*

- Datum: 15-11-2016 en 22-11-2016
- Gestelde vragen en antwoorden:

Project proposal:
-3.4.3 De onderzoekers zijn het eens met de commissie dat het 'proof of concept' moet zijn aangetoond voordat er aan zo een omvangrijke studie kan worden begonnen. Zij menen dat dit proof of concept al door ander onderzoek is aangetoond, maar dit betreft humane studies en muizenstudies. De gepresenteerde data zijn onvoldoende bewijs voor de toepasbaarheid van de neurotrofe hypothese bij de rattenmodellen die in deze projectaanvraag gebruikt zullen worden. De commissie meent dat het onderzoeken van de mechanismen inclusief de [redacted]' pas relevant is wanneer het [redacted] op het ontstaan van n[redacted]n optreedt in de diermodellen die de onderzoekers willen gebruiken. Zij verzoekt de onderzoekers nogmaals een extra go/ no go moment in te voeren met heldere criteria die duidelijk omschrijven wanneer zij de [redacted] **in deze modellen** afdoende bewezen achten.
Antwoord: Er zal een extra go / no-go moment worden toegevoegd zodat per diermodel, [redacted] *, eerst geconstateerd zal worden of het neurotrofe effect van* [redacted] *ook in het diermodel aanwezig is. Dus, mocht voor 1 van de 2 diermodellen de* [redacted] *niet worden aangetoond dan zal dit diermodel een no-go krijgen; dit is onafhankelijk van het andere diermodel. Hieronder en in het nieuwe projectvoorstel is om deze reden een pilot studie beschreven.*
Zoals eerder beschreven is er al humane en muizen data beschikbaar welke de invloed van [redacted] *hebben aangetoond op* [redacted] *. Dit geeft weer dat* [redacted] *op meerdere levels kunnen voorkomen en kunnen worden bestudeerd. Belangrijk te noemen is dat humane en muizen studies aan elkaar gerelateerd zijn gezien beiden een verandering hebben waargenomen in de somatosensorische cortex* [redacted] *). Hierom is verwacht dat in ratten deze maternaal serotonerge neurotrofe effecten ook plaatsvinden.*

Pilot studies
Er zullen twee pilot studies worden uitgevoerd, 1 kijkend naar de maternaal serotonerge effecten gevonden in muizen studies en 1 kijkend naar de [redacted] *gevonden in humane studies. Wanneer ten minste in 1 van deze pilot studies een significant verschil wordt aangetoond zal er een 'go' worden gegeven om verder te gaan met het onderzoek.*
In muizenstudies is aangetoond dat [redacted] *levels in het brein van de foetus verschilt n.a.v.* [redacted] *. Muller en collega's hebben dit jaar aangetoond dat op embryonaal dag (E) 14.5 pups van* [redacted] *(diermodel voor autistisch-gerelateerd gedrag)* [redacted] *muizen lagere* [redacted] *levels in hun voorbrein hebben t.o.v. pups van* [redacted]

[redacted]). Kijkend naar de gevolgen voor [redacted] constateerde ze een verbreding van de [redacted]-gevoelige thalamocortical axonen projecties. Deze projecteren onder andere naar de somatosensorische cortex.
Door dezelfde methode te handhaven willen wij in onze rattenmodellen aantonen of de [redacted] levels tijdens de embryonale fase wordt beïnvloed door het [redacted] en welke gevolgen dit heeft voor de [redacted] Mocht er een significant verschil zijn in de [redacted] levels en in de [redacted] dan is dit het bewijs dat [redacted] de foetus beïnvloed en zal er een go-moment worden gegeven om te onderzoeken of deze neurotrofe hypothese gerelateerd is aan de ontwikkeling van [redacted].
Methode: Embryonaal onderzoek E 10: [redacted] decapiteren en [redacted] levels in [redacted] van de pups onderzoeken d.m.v. HPLC en verschillende [redacted] bestuderen in geperfuseerde foetus breintjes d.m.v. immunohistochemistry.

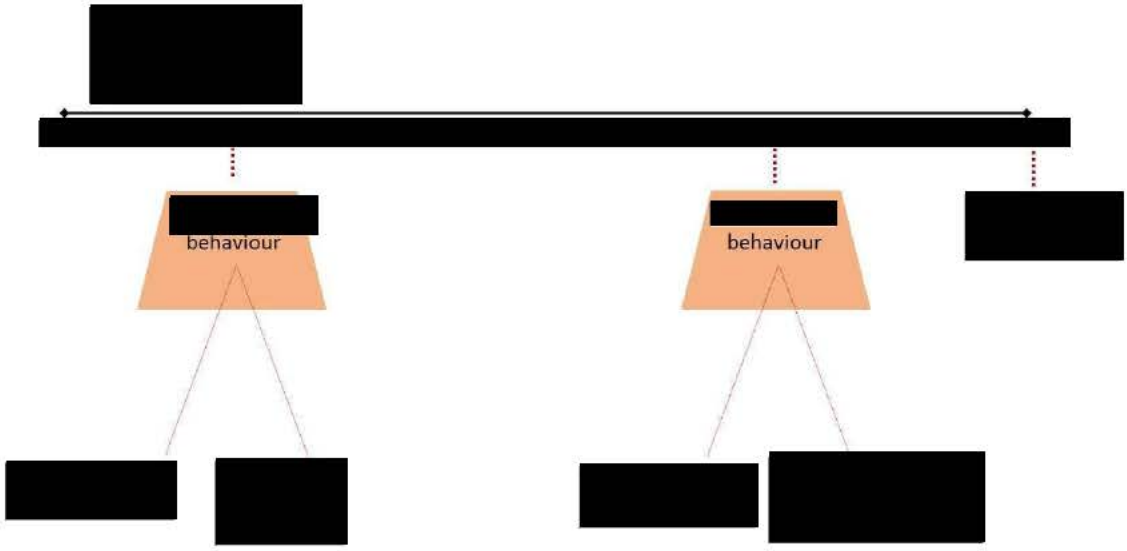
In humane studies is aangetoond dat het [redacted] het gedrag en de hersenontwikkeling beïnvloedt en legt daarbij een link met autisme. [redacted] hebben in 2014 laten zien dat het [redacted] geassocieerd is met een verhoogde somatosensorische functie en een grotere dichtheid in grijze stof van de somatosensorische cortex bij kinderen met het [redacted].
Deze data, te samen met de muizen data van [redacted]), suggereert een verband tussen [redacted] en de somatosensorische cortex (structuur en functie). De somatosensorische functie kan in dieren getest worden met de robotic gap crossing taak. Eerdere data heeft al aangetoond dat [redacted] ratten een beterde somatosensorische functie hebben t.o.v. [redacted] ratten waren sneller in het succesvol lokaliseren van de target en hadden hiervoor minder tactiele informatie nodig ([redacted]).
Gezien er via deze studies een verband wordt gelegd tussen het ([redacted] en deze cortex vaak wordt geassocieerd met autisme willen wij in deze pilot ook andere autistisch-gerelateerde gedragingen bestuderen. Eerder studies hebben laten zien dat [redacted] ratten en wistar ratten welke [redacted], andere gedragingen vertonen in de negative geotaxis, olfactory discrimination en social play testen zie tabel.

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted] de
[redacted]	[redacted]	[redacted]

In deze pilot studie [redacted] resulteert in veranderingen in gedrag of hersen structuur/functie welke overeenkomt met de bovenstaande data. Mocht er een significant verschil zijn in gedrag of hersen functie/structuur dan is dit het bewijs dat [redacted] de [redacted] beïnvloed en zal er een go-moment worden gegeven om te onderzoeken

of deze [redacted] hypothese gerelateerd is aan de [redacted]

Methode: 15[redacted]) zullen bloot worden gesteld aan bovenstaande gedragstaken op de leeftijd van PND 14 en 35; zie figuur. Na de laatste gedragstaken zullen de dieren geofferd worden en zal er in de hersenen gekeken worden naar veranderingen in structuur/functie, waaronder de structuur van de somatosensorische cortex. Mocht er cross-fostering moeten plaatsvinden zal het aantal dieren moeten worden verdubbeld naar 30[redacted] pups per groep zodat 15 pups gescoord worden terwijl deze bij hun eigen moeder zijn opgegroeid en 15 pups gescoord worden terwijl deze bij een foster-moeder zijn[redacted]



Criteria

Significant result [redacted]	*Significant result* [redacted] *experiment*	*Significant result* [redacted] *experiment*
[redacted] *e levels in het voorbrein van de foetus van* [redacted] *of* [redacted] *moeders is significant verschillend ten opzichte van de* [redacted] *levels in het voorbrein van de foetus van* [redacted] *of* [redacted] *moeders*	*Het gedrag van de HET pups van* [redacted] *of* [redacted] *moeders is significant verschillend ten opzichte van het gedrag van de HET pups van* [redacted] *of* [redacted] *moeders in ten minste 1 van de volgende gedragstaken* - *Negative geotaxis* - *Olfactory discrimination*	*De hersen structuur en/of functie van de HET pups van* [redacted] *of* [redacted] *moeders is significant verschillend ten opzichte van de hersen structuur en/of functie van de HET pups van* [redacted] *moeders in ten minste 1 van de volgende hersenstructuren* - *Somatosensory cortex (barrel fields)*

2. [redacted] *van* [redacted] *verschillend ten opzichte van de* [redacted] *in ten minste* [redacted]

-3.2: De tekst is hier essentieel veranderd (3 in plaats van 4 research questions) zonder dat dit duidelijk is aangegeven en toegelicht in de aanbiedingsbrief. De commissie vraagt zich af of dit ook op andere plaatsen is gebeurd. De commissie verzoekt de onderzoekers alle aanpassingen in het project t.o.v. de eerste versie te benoemen in de aanbiedingsbrief en te markeren in de projectaanvraag.

Antwoord: Mijn excuses, dit is op andere plekken niet gebeurd. [redacted] *hebben er uiteindelijk voor gekozen om de eerste twee onderzoeksvragen samen te voegen gezien beide vragen kijkt naar de invloed van* [redacted] *(hoog of laag level) op het gedrag en hersen structuur/functie. De inhoud is hierom niet veranderd, alleen het aantal vragen is aangepast naar de juiste hoeveelheid.*

1. Which ADHD/autism-related behavioral, brain structural & functional traits are caused by extracellular [redacted]

2. Which ADHD/autism-related behavioral, brain structural & functional traits are caused by extracellular [redacted]

Dit is nu: 3) What are the effects of [redacted] on offspring's measures of brain structure and function as well as the cognition and behavior of the pups?

Daarnaast is er gekozen voor een verandering in de volgorde van de onderzoeksvragen om deze op de juiste volgorde te zetten van het beloop van de [redacted]; dit is ook weergegeven in de figuur onder de vragen. Voor een goed verloop in de aanvraag is deze volgorde ook gebruikt in de rest van het project proposal en in de Description of animal procedures. Dit betekent dat de voormalig genoemde studie/DAP 1 en 2 sinds de vorige versie zijn omgedraaid t.o.v. de eerst ingediende versie.

Description of Animal Procedures:

-B: De commissie heeft de onderzoekers gevraagd of het aantal dieren beperkt kan worden door niet alle experimenten met zowel mannen als vrouwen te doen. Zij is het eens met de onderzoekers dat het efficiënter is om dieren van beide geslachten direct in plaats van sequentieel te gebruiken. De DEC meent echter dat het doel van het onderzoek mogelijk ook met minder dieren is te bereiken door gemengde groepen van iets grotere omvang (vanwege de grotere variatie) te nemen. Zij verzoekt de onderzoekers deze optie in hun reactie voor de DEC te evalueren en zonodig de benodigde aantallen dieren opnieuw te berekenen.
Antwoord: Allereerst bedankt voor het meedenken met de opzet van de proef. [redacted] zijn het er mee eens dat het van belang is om het aantal dieren zoveel mogelijk te beperken, echter is het niet gewild om gemengde groepen te gebruiken gezien het dan of onduidelijk is bij welk geslacht het effect wordt gevonden, of dat er geen effect wordt gevonden doordat de geslachtsverschillen de effecten van elkaar opheffen. Om deze reden verzoeken wij om de experimenten in beide geslachten apart maar wel gelijktijdig uit te voeren.
Nog een belangrijke reden om beide geslachten mee te nemen is dat depressie vaker voorkomt bij vrouwen, terwijl, zoals eerder aangegeven, ADHD en autisme juist vaker bij mannen voorkomt. In de [redacted] ratten is ook al eerder aangetoond dat angst-gerelateerd gedrag meer in vrouwen ratten is te zien ten opzichte van mannetjes ratten [redacted]., [redacted] Dit duidt nogmaals op het belang van het gebruik van beide geslachten.
Door tijdens het tweede go / no-go moment te bekijken welke studies significante verschillen aantoonden en met welk geslacht is er een mogelijkheid dat een studie met significante verschillen maar met 1 geslacht uitgevoerd zal worden.
-J: Bij de inschatting van de incidentie van humane eindpunten is per ongeluk blijven staan dat dit een fok zonder ongerief is.
Antwoord: Mijn excuus, dit is gewijzigd in een fok met ongerief.

Niet-technische samenvatting:

-De onderzoekers worden verzocht te checken of de beantwoording van bovenstaande vragen over het Project Proposal en de DAPs ook leidt tot aanpassingen in de NTS.
Antwoord: De geschatte dieraantallen en percentages verwachte ernst zijn in de NTS aangepast naar aanleiding van alle wijzigingen.

10. Eventuele adviezen door experts (niet lid van de DEC):
    - Aard expertise
    - Deskundigheid expert
    - Datum verzoek
    - Strekking van het verzoek
    - Datum expert advies
    - Advies expert

B. Beoordeling (adviesvraag en behandeling)

1. Het project is vergunningplichtig (dierproeven in de zin der wet).
2. De aanvraag betreft een nieuwe aanvraag.
3. De DEC is competent om hierover te adviseren.
4. Er is geen betrokkenheid van DEC-leden bij deze projectaanvraag, waardoor onafhankelijkheid en onpartijdigheid zijn gewaarborgd.

C. Beoordeling (inhoud)

1. Deze aanvraag heeft een concrete doelstelling en kan getypeerd worden als een project. De opzet komt het best overeen met voorbeeld 1 uit de handreiking 'Invulling definitie project' van de CCD. Het is helder welke handelingen individuele dieren zullen ondergaan. Hierdoor is ook duidelijk welk ongerief individuele dieren zullen ondergaan. De aanvrager heeft, zowel binnen de doelstellingen en bijlagen dierproeven, als tussen de doelstellingen, beschreven op basis van welke criteria zij zal besluiten het project wel of niet te continueren. De DEC is er daardoor van overtuigd dat de aanvrager gedurende het project op zorgvuldige wijze besluiten zal nemen over de voortgang van het project en er niet onnodig dieren gebruikt zullen worden.

2. Voor zover de DEC weet is er geen tegenstrijdige wetgeving die het uitvoeren van de proef in de weg zou kunnen staan.

3. De in de aanvraag aangekruiste doelcategorie is in overeenstemming met de hoofddoelstelling.

   Belangen en waarden

4. Het directe doel van het project is onderzoeken op welke manier [redacted] tijdens de dracht van invloed is op het ontstaan van [redacted] bij de nakomelingen. Het uiteindelijke doel is het ontwikkelen van nieuwe interventies tijdens de vroege zwangerschap om [redacted] te voorkomen of te verminderen. De onderzoekers zullen uitvoerig onderzoeken op welke manier [redacted] de hersenontwikkeling van embryo's kan beïnvloeden. Het is aannemelijk dat dit in mensen op vergelijkbare wijze gebeurt. De DEC is van mening dat er binnen dit project een reële relatie is tussen het directe doel en het uiteindelijke doel. Voorts is de DEC van mening dat het directe doel gerechtvaardigd is binnen de context van het onderzoeksveld.

5. De belangrijkste belanghebbenden in deze projectaanvraag zijn de proefdieren, de onderzoekers en de doelgroep/patiënten.
   Voor de proefdieren geldt dat hun welzijn en integriteit worden aangetast. De dieren zullen beperkt worden in hun natuurlijke gedrag en gedurende de proeven zullen de dieren stress ondervinden en pijn ondergaan. Uiteindelijk zullen ze in het kader van het onderzoek gedood worden. De dieren hebben er belang bij hiervan gevrijwaard te blijven.
   Voor de onderzoekers geldt dat het publiceren van belangrijke nieuwe wetenschappelijke inzichten resulteert in een goede wetenschappelijke reputatie, hetgeen vaak de sleutel is voor het verkrijgen van nieuwe onderzoeksmogelijkheden. Carrièremogelijkheden en welstand kunnen door de onderzoeker zelf van belang geacht worden, maar dienen naar de mening van de DEC geen rol te spelen in de ethische afweging over de toelaatbaarheid van het gebruik van proefdieren. Het gaat uiteindelijke om de vraag of dit onderzoek belangrijke maatschappelijke en wetenschappelijke doelen dient (gezondheid, kennis). Er dient tenminste (ook) sprake te zijn van een algemeen of publiek belang, wil een dierproef gerechtvaardigd zijn.

Voor patiënten is dit onderzoek indirect van belang, omdat het kan bijdragen aan een verbetering van hun geestelijke gezondheid en kwaliteit van leven. Gerichte preventie op basis van mechanistisch inzicht kan bijdragen aan het voorkomen of verminderen van [redacted] Dit kan er toe leiden dat kinderen zich normaal kunnen ontwikkelen, dan wel een betere kwaliteit van leven hebben. Het voorkomen of verminderen van neuro-ontwikkelingsstoornissen zoals ADHD en autisme, is van groot belang voor de samenleving.

6. De onderzoekers maken gebruik van transgene dieren waarbij zij de nationale GGO-regels in acht nemen. Hierdoor is er geen sprake van belangwekkende milieueffecten.

Proefopzet en haalbaarheid

7. De kennis en kunde van de onderzoeksgroep en andere betrokkenen bij de dierproeven zijn voldoende gewaarborgd. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager, zoals blijkt uit de in de aanvraag vermelde publicaties van deze onderzoeksgroep. De aanvragers beschikken over voldoende kennis en kunde om te kunnen voldoen aan alle zorgvuldigheidseisen omtrent het verrichten van dierproeven.

8. De doelstellingen van het project zijn realistisch en de voorgestelde experimentele opzet en uitkomstparameters sluiten hier logisch bij aan. Bovendien heeft deze groep heeft veel ervaring in dit onderzoeksveld en met de voorgestelde dierproeven. De DEC is dan ook van mening dat het project goed is opgezet, en dat deze strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project.

Welzijn dieren

9. Er is geen sprake van de volgende bijzonderheden op het gebied van categorieën van dieren, omstandigheden of behandeling van de dieren:
   ☐ Bedreigde diersoort(en) (10e lid 4)
   ☐ Niet-menselijke primaten (10e)
   ☐ Dieren in/uit het wild (10f)
   ☐ Niet gefokt voor dierproeven (11, bijlage I richtlijn)
   ☐ Zwerfdieren (10h)
   ☐ Hergebruik (1e lid 2)
   ☐ Locatie: buiten instelling vergunninghouder (10g)
   ☐ Geen toepassing verdoving/pijnbestrijding (13)
   ☐ Dodingsmethode niet volgens bijlage IV richtlijn (13c lid 3)

10. De huisvesting en verzorging van de dieren zijn conform de eisen in bijlage III van richtlijn 2010/63/EU.

11. Het cumulatieve ongerief als gevolg van de dierproeven is realistisch ingeschat en geclassificeerd. Het ongerief wordt hoofdzakelijk bepaald door de gedragstesten. Voor 70% van de dieren zal het ongerief licht zijn, voor de overige dieren matig.

12. De integriteit van dieren is aangetast doordat zij genetisch gemodificeerd zijn. Als gevolg hiervan is hun [redacted]-huishouding verstoord, hetgeen kan leiden tot licht afwijkend gedrag ten opzichte van [redacted] dieren. Bij één van de gebruikte diermodellen kunnen op latere leeftijd mogelijk hartproblemen optreden waardoor zij minder goed kunnen functioneren.

13. De criteria voor humane eindpunten zijn voldoende specifiek gedefinieerd en toegesneden op het experiment. Het percentage dieren dat naar verwachting een humaan eindpunt zal bereiken is op basis van eerdere ervaring met deze diermodellen en de beschreven handelingen ingeschat. Men verwacht dat [redacted] ratten een vergelijkbaar fenotype zullen hebben als [redacted] Het gehanteerde humane eindpunt is hierop gebaseerd. De commissie is het eens met deze inschatting en de gehanteerde humane eindpunten.

3V's

14. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn. [redacted] kunnen niet goed bestudeerd worden met proefdiervrije alternatieven. Het gedragsrepertoire van een rat is uitgebreid genoeg om deze ontwikkelingsstoornissen te kunnen onderzoeken. Om ethische redenen is het niet mogelijk dit onderzoek bij mensen uit te voeren.

15. Het maximale aantal te gebruiken dieren is realistisch ingeschat en is proportioneel ten opzichte van de gekozen onderzoeksopzet en de looptijd. De onderzoekers hanteren een goede strategie om ervoor te zorgen dat er met het kleinst mogelijke aantal dieren wordt gewerkt waarmee nog een wetenschappelijk betrouwbaar resultaat kan worden verkregen. Door de stapsgewijze aanpak waarin de resultaten uit eerdere proeven worden gebruikt voor het design van vervolgexperimenten wordt onnodig gebruik van proefdieren voorkomen. De commissie heeft met de aanvragers gediscussieerd over het inbouwen van een belangrijk go / no go moment aan het begin van dit onderzoek, en de noodzaak om dit onderzoek zowel in mannelijke als in vrouwelijke dieren apart uit te voeren. De commissie is er van overtuigd dat de onderzoekers hierin goede keuzes zullen maken waardoor er niet onnodig dieren worden gebruikt.

16. Het project is in overeenstemming met de vereiste van de verfijning van dierproeven. Er is zoveel mogelijk gekozen voor gedragstesten die licht ongerief voor de dieren veroorzaken. Enkele gedragstesten veroorzaken matig ongerief, maar zijn noodzakelijk om belangrijke gedragskenmerken te kunnen meten. De DEC is ervan overtuigd dat de beschreven proefopzet de meest verfijnde is en dat de dierproeven zo humaan mogelijk worden uitgevoerd.

17. Het betreft geen wettelijk vereist onderzoek.

Dieren in voorraad gedood en bestemming dieren na afloop proef

18. De vraagstelling wordt in mannelijke en vrouwelijke dieren apart onderzocht. Wanneer uit eerdere experimenten blijkt dat een effect slechts in één van beide sexen optreedt, dan worden vervolgexperimenten alleen met die sexe uitgevoerd. De pilotexperimenten worden alleen met mannelijke dieren gedaan omdat hiermee het optreden van de neurotrofe hypothese in deze diermodellen bevestigd kan worden en dit effect bij mannelijke dieren het grootst zal zijn. Op deze manier zullen minder dieren nodig zijn dan wanneer zowel mannelijke als vrouwelijke dieren hiervoor gebruikt worden. De DEC is van mening dat de aanvrager deze aanpak in voldoende mate wetenschappelijk heeft onderbouwd.

19. De dieren zullen in het kader van het project gedood worden. Dit is noodzakelijk om verschillende weefsels te kunnen onderzoeken voor het beantwoorden van bepaalde onderzoeksvragen. De gebruikte dodingsmethode staat vermeld in bijlage IV van richtlijn 2010/63/EU.

20. Er worden in deze projectaanvraag geen landbouwhuisdieren, honden, katten of niet-humane primaten gedood om niet-wetenschappelijke redenen.

    NTS

21. De niet-technische samenvatting is een evenwichtige weergave van het project en begrijpelijk geformuleerd.

D. Ethische afweging

1. Rechtvaardigt het belang van de doelstelling van het project het ongerief dat de dieren wordt aangedaan, en is aan alle zorgvuldigheidseisen (3V's) voldaan?

2. Er vindt een lichte of matige aantasting van welzijn en integriteit van de proefdieren plaats (beschreven in C9 tot C20). De doelstellingen kunnen niet zonder dieren behaald worden. De onderzoekers doen al het mogelijke om het lijden van de dieren en het aantal dieren te beperken.
   Voor de doelgroep/patiënten is dit onderzoek van belang, omdat het kan bijdragen aan een verbetering van hun geestelijke gezondheid en kwaliteit van leven. De DEC kent daar veel gewicht aan toe om de volgende redenen. [redacted] zoals ADHD en autisme zijn chronische aandoeningen die een grote impact hebben op patiënten en hun naasten tijdens alle levensstadia. De bestaande behandelingen zijn niet altijd effectief genoeg of hebben vervelende bijwerkingen. De resultaten van dit project zullen bijdragen aan de ontwikkeling van nieuwe interventies om ontwikkelingsstoornissen te voorkomen of te verminderen. Het is aannemelijk dat de doelstellingen op termijn behaald zullen worden. De commissie acht het voorkomen of verminderen van [redacted] van substantieel belang.

3. De DEC is overtuigd van het belang van de doelstellingen: onderzoeken op welke manier [redacted] van invloed is op het ontstaan van [redacted] bij ratmodellen, om uiteindelijk nieuwe interventies tijdens de vroege zwangerschap bij mensen te kunnen uitvoeren om [redacted] te voorkomen of te verminderen. De DEC is van mening dat de belangen van de patiënten voldoende zwaar wegen om het schaden van de belangen van de proefdieren (om gevrijwaard te blijven van een aantasting van hun welzijn en integriteit) te rechtvaardigen. De commissie is overtuigd van de kwaliteit van het werk van de aanvrager. De DEC is van mening dat het project goed is opgezet, en dat de gekozen strategie en experimentele aanpak kunnen leiden tot het behalen van de doelstelling binnen het kader van het project. De aanvrager heeft voldoende aannemelijk gemaakt dat er geen geschikte vervangingsalternatieven zijn, dat het doel niet met minder dieren behaald kan worden, dat de gebruikte aanpak de meest verfijnde is en dat zij zal kunnen voorkomen dat mens, dier en het milieu onbedoelde negatieve effecten ondervinden als gevolg van de dierproeven.
   De DEC is van oordeel dat het hier boven geschetste belang de onvermijdelijke nadelige gevolgen van dit onderzoek voor de dieren, in de vorm van angst, pijn of stress, rechtvaardigt. Aan de eis dat het belang van de experimenten op dient te wegen tegen het ongerief dat de dieren wordt berokkend, is voldaan.

E. Advies

1. Advies aan de CCD
   ☒ De DEC adviseert de vergunning te verlenen

☐ De DEC adviseert de vergunning te verlenen onder de volgende voorwaarden

- Op grond van het wettelijk vereiste dient de projectleider bij beëindiging van het project een beoordeling achteraf aan te leveren die is afgestemd met de IvD.
- Voor de uitvoering van dit project is tevens ministeriële ontheffing vereist
- Overige door de DEC aan de uitvoering verbonden voorwaarden, te weten...

☐ De DEC adviseert de vergunning niet te verlenen vanwege:

- De vaststelling dat het project niet vergunningplichtig is om de volgende redenen:…
- De volgende doorslaggevende ethische bezwaren:…
- De volgende tekortkomingen in de aanvraag:…

2. Het uitgebrachte advies is gebaseerd op consensus.

3. Er zijn geen knelpunten of dilemma's geconstateerd – zowel binnen als buiten de context van het project - die de verantwoordelijkheid en competentie van de DEC overstijgen.

Beste [redacted]

Op 29 december 2016 heeft u onze aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om het project [redacted] matters! [redacted] effect" met aanvraagnummer AVD103002017806. Gisteren heeft u laten weten dat u nog wat onduidelijkheden heeft gevonden in onze aanvraag betrekking hebbend op het aantal dieren.

Allereerst verwijst u naar bijlage 3.4.4.1. bij 'Pilot [redacted] Study'. In deze tabel staat bij een aantal proefgroepen n=7 hier wordt echter bij 'het totaal aantal dieren per groep' 4 dieren vermeld. Het aantal dieren per groep moet inderdaad 4 zijn, mijn excuses dat het niet overal goed is opgeschreven. Zie hieronder de aangepaste tabel:

PILOT [redacted] STUDY

[redacted]
1. [redacted] per group [redacted]
2. [redacted] per group [redacted]

[redacted]
1. [redacted] per group [redacted]
2. [redacted] per group [redacted]

[redacted] therefore, [redacted] need to be counted as well:
- [redacted] per group [redacted]
- [redacted] per group [redacted]

TOTAL PILOT STUDY: 24

Daarnaast geeft u aan dat de aantallen in de tabel in bijlage 3.4.4.3. (pagina 64/71) niet te herleiden zijn uit de aanvraag. Het is [redacted] in eerste instantie ontgaan dat tijdens het invoeren van deze aantallen de cijfers achter de punt niet zijn overgenomen. Dit heeft ervoor gezorgd dat alle aantallen boven de 1.000 werden afgerond naar de cijfer voor de punt. Hiervoor [redacted] excuses. Hieronder staan de juiste aantallen weergegeven met een uitleg hoe [redacted] aan deze aantallen zijn gekomen.

Rat	[redacted]	2.160	p1 – p157
Rat		20	adult
Rat		2.160	p1 – p157
Rat		1.020	adult

[redacted] **p1-p157:**
Behavioral studies, study 1: 600
Brain studies, study 1: 480
Behavioral studies, study 2: 600
Brain studies, study 2: 480
= 2.160 dieren

[redacted] **adult:**
[redacted] study 1: 10
study 2: 10

= 20 dieren

[redacted] **p1-p157:**
Behavioral studies, study 1: 600
Brain studies, study 1: 480
Behavioral studies, study 2: 600
Brain studies, study 2: 480
= 2.160 dieren

[redacted] **adult:**
[redacted] study 1:20
[redacted] study 1: 10
Breeding, study 1: 390
[redacted] study 2:20
[redacted] study 2: 10
Breeding, study 2: 570
= 1.020 dieren

Deze aanpassingen zijn doorgevoerd in de nieuwe versie van de Description Animal Procedure document [redacted]), zie bijlage.

Met vriendelijke groet,

[redacted]
PhD student

Centrale Commissie Dierproeven

> Retouradres Postbus 20401 2500 EK Den Haag

Stichting Katholieke Universiteit Nijmegen
[redacted]
Postbus 9101
6500 HB NIJMEGEN
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Postbus 20401
2500 EK Den Haag
centralecommissiedierproeven.nl
0900 28 000 28 (10 ct/min)
info@zbo-ccd.nl

Onze referentie
Aanvraagnummer
AVD103002016806
Bijlagen
1

Datum 20 februari 2017
Betreft Beslissing aanvraag projectvergunning Dierproeven

Geachte [redacted],

Op 29 december 2016 hebben wij uw aanvraag voor een projectvergunning dierproeven ontvangen. Het gaat om uw project "[redacted] matters [redacted] effect" met aanvraagnummer AVD103002016806. Wij hebben uw aanvraag beoordeeld.

Op 16 februari 2017 heeft u uw aanvraag aangevuld. Wij hebben u om aanvullende informatie gevraagd over het aantal dieren. Wij kunnen ons vinden in uw aanvulling.

Beslissing
Wij keuren uw aanvraag goed op grond van artikel 10a van de Wet op de Dierproeven (hierna: de wet). Hierbij gelden de voorwaarden zoals genoemd in de vergunning.
Met het oog op artikel 10a, lid 1, zijn er algemene voorwaarden gesteld.

U kunt met uw project [redacted] matters! [redacted] effect" starten. De vergunning wordt afgegeven van 22 februari 2017 tot en met 28 januari 2022.

Overige wettelijke bepalingen blijven van kracht.

Procedure
Bij uw aanvraag heeft u een advies van de Dierexperimentencommissie RU DEC gevoegd. Dit advies is opgesteld op 29 december 2016. Bij de beoordeling van uw aanvraag is dit advies betrokken overeenkomstig artikel 10a, lid 3 van de wet.
Wij kunnen ons vinden in de inhoud van het advies van de

Dierexperimentencommissie. Dit advies van de commissie nemen wij over, inclusief de daaraan ten grondslag liggende motivering. Er worden aanvullende algemene voorwaarde(n) gesteld.
Het DEC-advies en de in de bijlage opgenomen beschrijving van de artikelen van de wet- en regelgeving zijn de grondslag van dit besluit.

Bezwaar
Als u het niet eens bent met deze beslissing, kunt u binnen zes weken na verzending van deze brief schriftelijk een bezwaarschrift indienen.
Een bezwaarschrift kunt u sturen naar Centrale Commissie Dierproeven, afdeling Juridische Zaken, postbus 20401, 2500 EK Den Haag.

Bij het indienen van een bezwaarschrift vragen we u in ieder geval de datum van de beslissing waartegen u bezwaar maakt en het aanvraagnummer te vermelden. U vindt deze nummers in de rechter kantlijn in deze brief.

Bezwaar schorst niet de werking van het besluit waar u het niet mee eens bent. Dat betekent dat dat besluit wel in werking treedt en geldig is. U kunt tijdens deze procedure een voorlopige voorziening vragen bij de Voorzieningenrechter van de rechtbank in de woonplaats van de aanvrager. U moet dan wel kunnen aantonen dat er sprake is van een spoedeisend belang.

Voor de behandeling van een voorlopige voorziening is griffierecht verschuldigd. Op http://www.rechtspraak.nl/Organisatie/Rechtbanken/Pages/default.aspx kunt u zien onder welke rechtbank de vestigingsplaats van de aanvrager valt.

Meer informatie
Heeft u vragen, kijk dan op www.centralecommissiedierproeven.nl. Of neem telefonisch contact met ons op: 0900 28 000 28 (10 ct/minuut).

Centrale Commissie Dierproeven
namens deze:

ir. G. de Peuter
Algemeen Secretaris

Bijlagen:
- Vergunning
  Hiervan deel uitmakend:
  - DEC-advies
  - Weergave wet- en regelgeving

Centrale Commissie Dierproeven

Projectvergunning

gelet op artikel 10a van de Wet op de Dierproeven

Verleent de Centrale Commissie Dierproeven aan

Naam:	Stichting Katholieke Universiteit Nijmegen
Adres:	Postbus 9101
Postcode en plaats:	6500 HB NIJMEGEN
Deelnemersnummer:	10300

deze projectvergunning voor het tijdvak 22 februari 2017 tot en met 28 januari 2022, voor het project "[redacted] matters! [redacted] effect" met aanvraagnummer AVD103002016806, volgens advies van Dierexperimentencommissie RU DEC. Er worden aanvullende algemene voorwaarde(n) gesteld.
De functie van de verantwoordelijk onderzoeker is [redacted]. Voor de uitvoering van het project is Instantie voor Dierenwelzijn verantwoordelijk.
De aanvraag omvat de volgende bescheiden:

1 een aanvraagformulier projectvergunning dierproeven, ontvangen op 29 december 2016
2 de bij het aanvraagformulier behorende bijlagen:
a Projectvoorstel, zoals ontvangen per digitale indiening op 29 december 2017;
b Niet-technische Samenvatting van het project, zoals ontvangen per digitale indiening op 29 december 2017;
c Advies van dierexperimentencommissie d.d. 29 december 2016, ontvangen op 29 december 2016.
d De aanvullingen op uw aanvraag, ontvangen op 16 februari 2017

Aanvraagnummer:
AVD103002016806

Naam proef	Diersoort/ Stam	Aantal dieren	Ernst	Opmerkingen
3.4.4.1. Pilot study				
	Ratten (Rattus norvegicus) /	164	100% Licht	
3.4.4.2. Identification of [redacted] and its [redacted] of [redacted]				Drachtige vrouwtjes en volwassen mannetjes: 210 Embryo's (E20): 364
	Ratten (Rattus norvegicus) /	574	100% Licht	
3.4.4.3. [redacted] traits				
	Ratten (Rattus norvegicus) /	5.360	36% Matig 64% Licht	

Voorwaarden

Op grond van artikel 10a1 lid 2 van de Wet op de dierproeven zijn aan een projectvergunning voorwaarden te stellen

De vergunning wordt verleend onder de voorwaarde dat go/no go momenten worden afgestemd met de IvD.

In artikel 10, lid 1 sub a van de wet, wordt bepaald dat het verboden is een dierproef te verrichten voor een doel dat, naar de algemeen kenbare, onder deskundigen heersende opvatting, ook kan worden bereikt anders dan door middel van een dierproef, of door middel van een dierproef waarbij minder dieren kunnen worden gebruikt of minder ongerief wordt berokkend dan bij de in het geding zijnde proef het geval is. Nieuwe onderzoeken naar alternatieven kunnen tot gevolg hebben dat inzichten en/of omstandigheden van

het aangevraagde project in de vergunningsperiode wijzigen, gedurende de looptijd van deze vergunning. Indien bovenstaande zich voordoet dient aanvrager dit in afstemming met de IvD te melden bij de CCD. De CCD kan in een dergelijke situatie aan de vergunning nieuwe voorwaarden verbinden en gestelde voorwaarde wijzigen of intrekken.

Aanvraagnummer:
AVD103002016806

Weergave wet- en regelgeving

Dit project en wijzigingen
Volgens artikel 10c van de Wet op de Dierproeven (hierna de wet) is het verboden om andere dierproeven uit te voeren dan waar de vergunning voor is verleend. De dierproeven mogen slechts worden verricht in het kader van een project, volgens artikel 10g. Uit artikel 10b volgt dat de dierproeven zijn ingedeeld in de categorieën terminaal, licht, matig of ernstig. Als er wijzigingen in een dierproef plaatsvinden, moeten deze gemeld worden aan de Centrale Commissie Dierproeven. Hebben de wijzigingen negatieve gevolgen voor het dierenwelzijn, dan moet volgens artikel 10a5 de wijziging eerst voorgelegd worden en mag deze pas doorgevoerd worden na goedkeuren door de Centrale Commissie Dierproeven.
Artikel 10b schrijft voor dat het verboden is een dierproef te verrichten die leidt tot ernstige mate van pijn, lijden, angst of blijvende schade die waarschijnlijk langdurig zal zijn en niet kan worden verzacht, tenzij hiervoor door de Minister een ontheffing is verleend.

Verzorging
De fokker, leverancier en gebruiker moeten volgens artikel 13f van de wet over voldoende personeel beschikken en ervoor zorgen dat de dieren behoorlijk worden verzorgd, behandeld en gehuisvest. Er moeten ook personen zijn die toezicht houden op het welzijn en de verzorging van de dieren in de inrichting, personeel dat met de dieren omgaat moet toegang hebben tot informatie over de in de inrichting gehuisveste soorten en personeel moet voldoende geschoold en bekwaam zijn. Ook moeten er personen zijn die een eind kunnen maken aan onnodige pijn, lijden, angst of blijvende schade die tijdens een dierproef bij een dier wordt veroorzaakt. Daarnaast zijn er personen die zorgen dat een project volgens deze vergunning wordt uitgevoerd en als dat niet mogelijk is zorgen dat er passende maatregelen worden getroffen.
In artikel 9 staat dat de persoon die het project en de dierproef opzet deskundig en bekwaam moet zijn. In artikel 8 van het Dierproevenbesluit 2014 staat dat personen die dierproeven verrichten, de dieren verzorgen of de dieren doden, hiervoor een opleiding moeten hebben afgerond.
Voordat een dierproef die onderdeel uitmaakt van dit project start, moet volgens artikel 10a3 van de wet de uitvoering afgestemd worden met de instantie voor dierenwelzijn.

Pijnbestrijding en verdoving
In artikel 13 van de wet staat dat een dierproef onder algehele of plaatselijke verdoving wordt uitgevoerd tenzij dat niet mogelijk is, dan wel bij het verrichten van een dierproef worden pijnstillers toegediend of andere goede methoden gebruikt die de pijn, het lijden, de angst of de blijvende schade bij het dier tot een minimum beperken. Een dierproef die bij het dier gepaard gaat met zwaar letsel dat hevige pijn kan veroorzaken, wordt niet zonder verdoving uitgevoerd. Hierbij wordt afgewogen of het toedienen van verdoving voor het dier traumatischer is dan de dierproef zelf en het toedienen van verdoving onverenigbaar is met het doel van de dierproef. Bij een dier wordt geen stof toegediend waardoor het dier niet meer of slechts in verminderde mate in staat is pijn te tonen, wanneer het dier niet tegelijkertijd voldoende verdoving of pijnstilling krijgt toegediend, tenzij wetenschappelijk gemotiveerd. Dieren die pijn

kunnen lijden als de verdoving eenmaal is uitgewerkt, moeten preventief en postoperatief behandeld worden met pijnstillers of andere geschikte pijnbestrijdingsmethoden, mits die verenigbaar zijn met het doel van de dierproef. Zodra het doel van de dierproef is bereikt, moeten passende maatregelen worden genomen om het lijden van het dier tot een minimum te beperken.

Einde van een dierproef
Artikel 13a van de wet bepaalt dat een dierproef is afgelopen wanneer voor die dierproef geen verdere waarnemingen hoeven te worden verricht of, voor wat betreft nieuwe genetisch gemodificeerde dierenlijnen, wanneer bij de nakomelingen niet evenveel of meer, pijn, lijden, angst, of blijvende schade wordt waargenomen of verwacht dan bij het inbrengen van een naald. Er wordt dan door een dierenarts of een andere ter zake deskundige beslist of het dier in leven zal worden gehouden. Een dier wordt gedood als aannemelijk is dat het een matige of ernstige vorm van pijn, lijden, angst of blijven schade zal blijven ondervinden. Als een dier in leven wordt gehouden, krijgt het de verzorging en huisvesting die past bij zijn gezondheidstoestand.
Volgens artikel 13b moet de dood als eindpunt van een dierproef zoveel mogelijk worden vermeden en vervangen door in een vroege fase vaststelbare, humane eindpunten. Als de dood als eindpunt onvermijdelijk is, moeten er zo weinig mogelijk dieren sterven en het lijden zo veel mogelijk beperkt blijven.
Uit artikel 13d volgt dat het doden van dieren door een deskundig persoon moet worden gedaan, wat zo min mogelijk pijn, lijden en angst met zich meebrengt. De methode om te doden is vastgesteld in de Europese richtlijn artikel 6.
In artikel 13c is vastgesteld dat proefdieren geadopteerd kunnen worden, teruggeplaatst in hun habitat of in een geschikt dierhouderijsysteem, als de gezondheidstoestand van het dier het toelaat, er geen gevaar is voor volksgezondheid, diergezondheid of milieu en er passende maatregelen zijn genomen om het welzijn van het dier te waarborgen.
De Minister heeft vrijstelling ontheffing verleend volgens artikel 13c, die de afwijkende methode van doden op basis van wetenschappelijke motivering ten minste even humaan acht als de in de richtlijn opgenomen passende methoden.